

2017

## 2017 GJMPP Monograph Series: Grace Jordan McFadden Professors Program

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# **GJMPP Monograph Series**

**Grace Jordan McFadden  
Professors Program**

**John McFadden, PhD**

**Executive Editor**

**University of South Carolina**

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## FOREWORD

I am honored and so humbled to have been given the opportunity to write the Foreword for the inaugural edition of the Grace Jordan McFadden Professors Program monograph, formerly the Carolina Diversity Professors Program (CDPP) and the African American Professors Program (AAPP). This annual publication is something so many look forward to each year as it displays the brilliance of current students enduring the doctoral process as well as showcasing the growth of the program's graduates. The monograph series symbolizes the vision that has always been dear to both Drs. John McFadden and Grace Jordan McFadden of cultivating young scholars and encouraging their light to shine throughout this world.

My journey at the University of South Carolina began as an undergraduate student in the College of Pharmacy. I knew that I did not want to pursue the standard route of pharmacy practice as most young pharmacists do.

Although there is nothing wrong with that route, I had an interest in different areas of research not pursued by many. I had spoken with the former dean of the College of Pharmacy about my interests in graduate studies. He encouraged me to pursue them as he and one of his daughters had done. Dr. Farid Sadik guided me through the application process to graduate school, advised me to check the box that I was interested in an assistantship and to identify the date to take the Graduate Record Exam (GRE). I did all of these things as instructed and completed them well and on time, which ensured that I would be able to enroll in the PhD program January 2001.

My graduation date from pharmacy school was December 18, 2000. It was a cold Monday morning, and several relatives had come from as far as New York for my graduation (i.e., My mother remembers it better than I do). While I was attending the reception held for the December PharmD graduates after the doctoral hooding ceremony in the Coker Life Sciences Lobby where the pharmacy program is housed, Dr. Sadik approached me. He told me that he had spoken on my behalf to Dr. John McFadden

and that I would be enrolled in AAPP in January. He cautioned me not to worry because arrangements were made for all of my fees to be covered. I was in such shock and so grateful that I had been blessed with such a wonderful dean who was able to contact his good friend and fellow higher education colleague to assist me in this new journey. My mother's eyes welled up as did mine that such a gift had come a week before Christmas. I had not heard of the African American Professors Program until that day, but forever I am indebted to it.

The African American Professors program (now the Grace Jordan McFadden Professors Program) has gifted me tremendously in so many ways that are too numerous to recount for this *Foreword*. The financial support alone is a blessing as a fulltime doctoral student, but more importance is placed on the lifelong friendships that I have retained that were fostered initially by the program. I met fellow scholars who looked like me that I may not have ever met if not for my AAPP participation and the leadership of Dr. McFadden as well as the love of Mrs. Rhittie Gettone. The workshops that prepared me for

future interviews (e.g., seeking postdoctoral fellowships and other employment opportunities) are invaluable. The genuine camaraderie AAPP fosters in the monthly meetings and scheduled programs served as not only a learning tool, but they also functioned as a social outlet. Because I was the only African American graduate student in my department as well as the only woman out of only four students, AAPP students provided needed support. As I grew in my program and within AAPP, I became a bit of a social butterfly scheduling monthly gatherings near campus or at my home where we could continue to get to know each other better; thus, the roots began for these lifelong friendships that I still hold dear and near.

Thanks to the foundation I received from AAPP I have been able to collaborate with other pharmacy scholars on a textbook since my graduation in May, 2005. I have served as the president of both my local and state African American Medical Societies. While at USC, I served as the chair of the Black Alumni Council and was featured in the Alumni spotlight thanks to my nomination by the Dean, Dr. Farid Sadik. I also was recognized in the

South Carolina Black Pages Inaugural Top 40 under 40, in the community category, in 2014.

I am forever grateful for the African American Professors Program and the support of numerous others: Dr. Farid Sadik, Dean Emeritus of the College of Pharmacy; Dr. Michael Dickson, Professor Emeritus of the College of Pharmacy and my dissertation chair; current GJMPP Director and The Benjamin Elijah Mays Distinguished Professor Emeritus, Dr. John McFadden; and the devoted GJMPP Administrative Coordinator, Mrs. Rhittie Gettone. In addition, my gratitude also is extended to: The University of South Carolina Office of the Provost for their continued AAPP/GJMPP sponsorship; the personal and professional networks formed through my involvement in the AAPP program; and scholars and faculty mentors who are featured in the Monograph Series and whose published works are both helpful and inspirational.

Congratulations to the Grace Jordan McFadden Professors Program Alumni featured in this Monograph Series, and I look forward to the continued learning I will

receive from this latest edition. God bless you as you continue your journey.

***Phyllis I. Perkins, PhD, PharmD, MBA***

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## **PREFACE**

The Grace Jordan McFadden Professors Program (GJMPP), formerly the African American Professors Program (AAPP)/Carolina Diversity Professors Program (CDPP) at the University of South Carolina, is honored to publish its sixteenth edition of this annual monograph series. AAPP recognizes the significance of offering its scholars a venue through which to engage actively in research and to publish their refereed papers that continually contribute to their respective fields of study. Parallel with the publication of their manuscripts is the opportunity to gain visibility among colleagues throughout postsecondary institutions at national and international levels.

Scholars who have contributed papers for this monograph are acknowledged for embracing the value of including this responsibility within their academic milieu. Writing across disciplines adds broadly to the intellectual

diversity of these manuscripts. From neophytes to quite experienced individuals, the chapters have been researched and written in depth.

Founded in 1997 through the Department of Educational Leadership and Policies in the College of Education, AAPP was designed originally to address the under-representation of African American professors on college and university campuses. Its mission is to expand the pool of these professors in critical academic and research areas. Sponsored historically by the University of South Carolina, the W. K. Kellogg Foundation, and the South Carolina General Assembly, the program recruits doctoral students for disciplines in which African Americans currently are underrepresented among faculty in higher education.

The continuation of this monograph series is seen as responding to a window of opportunity to be sensitive to an academic expectation of graduates as they pursue career placement and, at the same time, to allow for the dissemination of products of scholarship to a broader community. The importance of this series has been voiced

by one of our 2002 AAPP graduates, Dr. Shundelle LaTjuan Dogan, formerly an Administrative Fellow at Harvard University, a Program Officer for the Southern Education Foundation, and a Program Officer for the Arthur M. Blank Foundation in Atlanta, Georgia. She is currently a Corporate Citizenship and Corporate Affairs Manager for IBM-International Business Machines in Atlanta, Georgia and has written an impressive Foreword for the 2014 monograph.

Dr. Dogan wrote: *“One thing in particular that I want to thank you for is having the African American Professors Program scholars publish articles for the monograph. I have to admit that writing the articles seemed like extra work at the time. However, in my recent interview process, organizations have asked me for samples of my writing. Including an article from a published monograph helped to make my portfolio much more impressive. You were ‘right on target’ in having us do the monograph series” (AAPP 2003 Monograph, p. xi).*

The Grace Jordan McFadden Professors Program purports to advance the tradition of spearheading international scholarship in higher education as evidenced through inspiration from this group of interdisciplinary manuscripts. I hope that you will envision these published papers for serving as an invaluable contribution to your own professional and career enhancement.

***John McFadden, PhD***

*The Benjamin Elijah Mays*

*Distinguished Professor Emeritus*

*Director, Grace Jordan McFadden Professors Program*

*University of South Carolina*

*Columbia, South Carolina*



**2017**

**ALUMNI CONTRIBUTIONS**

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**SCHOOL RACIAL CLIMATE: A BRIEF REVIEW  
EXPLORING AFRICAN AMERICAN YOUTHS'  
PERCEPTIONS, ASSOCIATIONS WITH THEIR  
EDUCATIONAL OUTCOMES, AND IMPLICATIONS  
FOR EDUCATORS' PRACTICE**

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School climate is a multidimensional construct that includes major spheres of school life such as students' sense of safety, relationships, teaching and learning, and organizational patterns (National School Climate, 2017). The quality of a school's climate is characterized by four levels of interactions: (a) interactions among students, (b) interactions between school personnel and students, (c) interactions among school personnel, and (d) interactions

between the school, families, and community (Richman, Bowen & Woolley, 2004). Scholars, practitioners, educators, and policymakers have acknowledged the significant role that school climate has on students' learning and development (e.g., Blum, McNeely & Nonnemaker, 2002; Kuperminc, Leadbeater, & Blatt, 2001; Loukas, Suzuki, & Horton, 2006; Ruus, Veisson, Leino, Ots, Pallas, Sarv, & Veisson, 2007; Whitlock, 2006). Indeed, empirical evidence suggests that a positive school climate is associated with academic achievement, effective risk prevention, and positive youth development (National School Climate Center, 2017). Despite the abundance of evidence demonstrating associations between school climate and student outcomes, there has been a dearth of research investigating the importance of school *racial* climate.

School racial climate describes how race and perceptions regarding race function within the school setting concerning values, practices and norms experienced by individuals within the environment (Mattison & Aber, 2007). Consistent with the multidimensional nature of the



broader school climate literature, researchers have recognized four re-emerging themes throughout school racial climate scholarship, which includes interpersonal interactions, stereotypes and race relations, fair treatment and racial equity, and support (Byrd, 2015; Byrd & Chavous, 2011; Hope, Skoog, & Jagers, 2015). Although researchers such as Chavous (2005; 2008) and Brody, et al. (2006) have contributed to understanding the consequences of school racial climate for adolescent development, this body of literature remains small and often oversimplified.

Even though professional literature on school racial climate among African American youth has been under-explored, current events around our nation highlight the need for the examination of how perceptions of race matter in schooling. For instance, according to the Department of Education and Justice, students of color “are more likely to be suspended and expelled when compared with their White peers,” and these differences in disciplinary practices cannot be explained by more frequent or more serious misbehavior by students of color (Lhamon & Samuels, 2014, p. 6).

Many racial and ethnic minority youth are faced with the challenge of navigating their schooling experience while also dealing with complex racial issues. Also, theoretical frameworks (e.g., the integrative model of ethnic identity) highlight how the effect of social position factors into school experience. Factors such as race include “nonshared” experiences with mainstream populations and help to “define the unique development of ethnic minority youth” (Garcia-Coll, et al., 1996, p. 1896). Specifically, this theoretical framework posits that considerations of race-related experiences should be central and not peripheral to ethnic minority youths’ outcomes (i.e., particularly in school contexts) because of the saliency of this environment in the development of ethnic minority children. In additional frameworks (e.g., the Spencer, Dupree, and Hartman [1997] phenomenological variant of ecological systems theory [PVEST]), African American children experience a unique ecology that is important for researchers to understand when examining developmental outcomes, including academic achievement.

The current review begins by providing a brief overview of the research literature exploring how African American youth perceive their school's racial climate. Next, we discuss associations between African American youths' perceptions of racial climate and varying outcomes. Finally, we focus our discussion on important implications for educators' practice.

## **YOUTHS' PERCEPTIONS OF SCHOOL RACIAL CLIMATE**

Surprisingly, racial climate has been the focus of several studies at the college level (e.g., Chavous, 2005), but only a few studies have been conducted at the secondary school level (e.g., Byrd & Chavous, 2011; Mattison & Aber, 2007). The Chavous (2005) research findings suggest that White students and ethnic minority students on college campuses often experience different racial climates. In general, White students tend to report more positive perceptions of intergroup relationships and diversity norms at their institutions than do African Americans (e.g., Ancis, Sedlacek, & Mohr, 2000).

Some research indicates that students may be similar in their views about particular aspects of the environment, but they may differ regarding other aspects of the racial climate. Loo and Rolison (1986), for instance, found that minority students (e.g., African Americans, Mexican Americans, and Asian Americans) and White students agreed about the existence of sociocultural difficulties among students; however, White students perceived that there were greater levels of university support for minorities than ethnic minority students perceived.

It may be to no surprise then, that students of color experience secondary school settings in ways that are notably distinct from their White peers (Fisher, Wallace, Fenton, 2000; Greene, Way, & Pahl, 2006; McLoyd & Steinberg, 1998; Rosenbloom & Way, 2004). Children as young as 5 years old can notice differential treatment across racial groups (Brown & Bigler, 2004), and children's understanding of racial inequality, prejudice and discrimination becomes increasingly complex with age (Brown & Bigler, 2004; Quintana & Vera, 1999). There have been several findings indicating that African

American youth perceive more racial discrimination than their Hispanic, Asian American, or White peers. For example, in a sample of 3,071 adolescents ranging in age from 10-17, Romero and Roberts (1998) found that African American youth perceived more discrimination than their European-American, Mexican-American, and Vietnamese-American counterparts. Research also indicates that African American youth experience this racially-biased treatment even in the context of schooling (e.g., Fisher, et al., 2000; Greene, et al., 2006; Rosenbloom & Way, 2004; Wong, Eccles, & Sameroff, 2003).

At school, African American adolescents are likely to have White teachers, even in predominantly Black schools (i.e., approximately 80% of U.S. high school teachers are White; U.S. Department of Education, 2016). Also, teachers in general are unlikely to have received extensive training in multicultural education (Ford & Kea, 2009; Ford & Harris, 1996; Hollins & Guzman, 2005); therefore, it is not surprising that African American youths report racially biased treatment within the classroom. Rosenbloom and Way (2004) used interviews and participant observations to

describe how ethnic minority students in an urban high school experienced discrimination. Findings indicated that when African American students were asked about their experiences with discrimination, they described hostile relationships with adults in positions of authority, which included teachers in their schools (Rosenbloom & Way, 2004).

Futhermore, African American students are more likely to contend with negative racial stereotypes from students and staff (Codjoe, 2001) such as being perceived by teachers as less intelligent (Aronson, Quinn, & Spencer, 1998; Chavous, Rivas-Drake, Smalls, Griffin, & Cogburn, 2008; Fisher, et al., 2000; Green, et al., 2006). In their study of African American adolescents, Hope, Skoog, and Jager (2015) revealed reports of active stereotypes throughout the school environment, including teacher expectations of poorer performance and more problematic behaviors for African American students. In another investigation among 177 high school youth, Fisher and colleagues (2000) noted that 32% of African Americans reported being discouraged from taking advanced level

courses and being treated unfairly in comparison to only 13% of the White youth in the sample. The Fisher and colleagues (2000) study also found that 46% of African Americans youth reported that they were given a lower grade than they deserved because of their race or ethnicity. Research also provides evidence that African American students are more likely to be disciplined harshly at school due to race (e.g., Chavous, et al., 2008; Cogburn, Chavous, & Griffin, 2011; Fisher, Wallace, & Fenton, 2000; Wong, et al., 2003). In the same study, Fisher and colleagues (2000) found that when compared with students from other ethnic groups, African American students, in particular, believed that racial discrimination resulted in being disciplined unjustly in school by teachers.

Similarly, research indicates that peer interactions at school represent an important milieu for adolescents (Anderman, 2002; Brown, 1990; Buhs & Ladd, 2001; DeRosier, Kupersmidt, & Patterson, 1994; Wentzel & Caldwell, 1997). Specifically, peer interactions may serve as a context in which minority youth experience racially motivated social exclusion or harassment (Scott, 2004;

Wong, et al., 2003). Previous research supports this assertion and indicates that adolescent youth perceive differential treatment from their same-age peers (DuBois, Burk-Braxton, Swenson, Tevendale, & Hardesty, 2002; Fisher, et al., 2000; Wong, et al., 2003). Peer racial discrimination manifests in multiple ways, including intentional or overt social exclusion, teasing, hitting, and unfair treatment based on ethnic group membership (Brown & Bigler, 2005; Chavous, et al., 2008; Greene, et al., 2006; Rosenbloom & Way, 2004; Verkuyten & Steenhuis, 2005; Wong, et al., 2003). The ethnic and racial hierarchies that emerge in classroom-based sociometric research (e.g., Graham, Taylor, & Hudley, 1998; Verkuyten & Kinket, 2000), in which ethnic minorities tend to have lower status and are ascribed less favorable characteristics when rated by peers, also implies that implicit negative group stereotypes operate within same age-peer groups. In addition to racial climate influencing student interactions, the following section explores its relationship to student academic outcomes.



## **RACIAL CLIMATE AND STUDENT OUTCOMES**

### **Academic Outcomes**

Though relatively few in number, published research has demonstrated that African American students experiencing school racial climates characterized by frequent intergroup contact, fair treatment, and respect for all races show more positive achievement (Green, et al., 1988; Mattison & Aber, 2007) and motivation (Brand, Felner, Shim, Seitsinger, & Dumas, 2003; Ryan & Patrick, 2001). The broad literature, which is focused on teacher-student relationships among adolescent youth, indicates that perceptions of strong teacher support and high expectations relate to higher self-efficacy, motivation efforts, and school performance among African American youth (e.g., Honora, 2003; Marcus, Gross, & Seefeldt, 1991). Conversely, ethnic minority youth perceiving a lack of such support and low expectations, particularly when based on their racial group, may be at risk for less positive academic outcomes (Irvine, 1986; Murdock, 1999; Roeser, Eccles, & Sameroff, 2000; Wong, et al., 2003). With regard to peer interactions, there is evidence of the negative

academic impact of general social rejection and peer harassment on African American youth (Graham & Juvonen, 2002; Spencer, Dupree & Hartman, 1997), as well as those interactions that are specifically due to race (DuBois, et al., 2002; Fisher, et al., 2000; Wong, et al., 2003).

A small but growing body of literature, which examines within-school racial discrimination experiences and academic adjustment among adolescent youth, also indicates that racial discrimination undermines success in the academic domain (e.g., Fisher, et al., 2000; Chavous, et al., 2008; Wong, et al., 2003). In particular, adolescents who perceive more discrimination have lower grades (Wong, et al., 2003), lower academic self-concept (Eccles, Wong, & Peck, 2006) and less academic curiosity and persistence (Fisher, et al., 2000) compared with those who perceive less discrimination or none at all. In a study of 334 African American high school students, Brown and Jones (2004) found that perceptions of bias within the school context were related to both lower academic values and academic utility (i.e., the usefulness of formal education).

Another finding from this investigation was that students who perceived that school and classroom practices were fair to all students were more likely to have positive educational outcomes. In one of the few studies to examine these relationships longitudinally, Wong and colleagues (2003) found that experiences with teacher and peer discrimination decreased achievement motivation and academic outcomes, while they also increased the likelihood of problem behaviors in African American adolescents. In addition, Okeke, Howard, Kurtz-Costes, and Rowley (2009) as well as other scholars (e.g., Steele & Aronson, 1998) have found evidence that negative racial stereotypes concerning academic skills negatively affected the students' beliefs about their academic ability. Taken together, this research provides some evidence that African American youths' perceptions of and experiences with the school's racial climate are associated with academic adjustment and also can be related to psychological and behavioral outcomes.

## **Psychological Outcomes and Behavioral Outcomes**

While much of the broader school climate literature has focused on associations with academic outcomes, there is some empirical evidence that school climate may be related to a range of psychological outcomes and behaviors. Much of this small body of work has explored associations between African American student experiences with racial discrimination from teachers and peers. For example, in a study exploring experiences with school-based racial discrimination from teacher and peers among African American middle school students, Cogburn, Chavous, and Griffin (2011) found that these negative interpersonal experiences were associated with higher depressive symptomology and lower self-esteem. In a multi-ethnic sample of adolescents, Greene, et al. (2006) also found that perceived discrimination from peers and adults was associated with lower self-esteem and increased depressive symptoms over a three-year period. Additional research has shown that perceptions of racial discrimination were linked not only to an increased number of depressive symptoms but also to an increased number of conduct problems over

time among rural African American youth (Brody, et al., 2006).

Regarding behavioral outcomes, the extant literature seems quite limited. In their investigation that explored associations between student perceptions of the broader school climate and office discipline referrals, Gage, Larson, Sugai, and Chafouleas (2016) found that positive school climate was associated with fewer office-discipline referrals and out-of-school suspensions. Additional empirical evidence also provides support for the predictive value of school climate in relation to behavior outcomes (e.g., Wang, 2009; Wang, Selman, Dishion, & Stormshak, 2010). Similar to the limitations in the broader school climate literature, empirical evidence demonstrating relationships between school racial climate and behavioral outcomes is much less abundant. To our knowledge, one of the first published studies to link school racial climate to students' discipline outcomes was conducted by Mattison and Aber (2009), using a sample of African American and European American students. Their results indicated that positive perceptions of the racial climate (as measured by

perceptions of racial fairness, experiences with racism, and the need for change) were associated with fewer detentions and suspensions for all youth in the sample that, of course, included African Americans.

## **IMPLICATIONS FOR EDUCATOR PRACTICE**

In general, there are a limited number of empirical studies specifically addressing school racial climate among African American youth (Griffin, Cooper, Metzger, Golden, & White, 2017); however, over the past several decades there have been numerous theoretical frameworks that emphasize the importance and impact of race in the schooling process of African American students (e.g., Aronson, et al., 1998; Mickelson, 1990; Ogbu, 1978, 1994; Osborne, 1995, 1997; Steele, 1992, 1997, 1999; Steele & Aronson, 1995).

Though these theoretical perspectives differ slightly, they have a common thread among them. They emphasize that the school-related outcomes of African American students are an interaction of their race-specific experiences

within the school setting (e.g., Mickelson, 1990; Ogbu, 1994). In totality, these theoretical frameworks regarding how race matters in schooling seem to indicate that racial differences in student outcomes also may derive from students' perceptions of school racial climate (e.g., Osborne; Steele, & Aronson, 1995). Taken together, both theory and empirical evidence support the conclusion that racial climate is associated with African American youths' school adjustment, and this literature holds several implications for educator practice that also can be considered in future research.

For educators, who are practicing full-time in school-buildings, this quite likely means that attention should be given to school-based prevention directed at school racial climate. This attention may include reaffirming the importance of creating school environments that are supportive and encouraging, with particular attention to racialized dynamics of the schooling process (Wang, Brinkworth, & Eccles, 2013). Teacher-training programs and school institutions would benefit from both acknowledging and actively safe guarding against racial

biases in key elements of practice, including teacher expectations, course placements, discipline practices and student–teacher interactions (Conchas & Rodriguez, 2007). More than 2 decades ago, Ladson-Billings (1995) concluded that teacher-preparation programs also should educate teachers as to how discriminatory and non-inclusive behaviors and bias transpire in the classroom as well as suggesting potential strategies for dealing with these issues, which is a position congruent with that of Conchas and Rodriguez (2007). In addition, helping teachers to become aware of their biases within the classroom today certainly has implications also for how students perceive and behave toward their fellow students of color.

In addition, students themselves need to understand the effects of how any form of peer harassment, including racial harassment, may impact their peers' schooling experience. Peers should learn to work individually and collectively to create social networks that are more open and accepting. In addition, educators can learn to teach these concepts to their students.



Clearly, by addressing these issues within schools, students can learn to be both conscientious and courageous in holding themselves and their peers accountable for creating a positive racial climate in school that also can benefit them in furthering their education and in the future world of work as well. In the end, positive school adjustment among African American youth will be determined in part by the collective response of parents, teachers, and students to the potential negative race-associated school experiences. In conclusion, multi-level intervention approaches targeting community factors may be needed as the school's racial climate may be tied to the history of the community in which the school exists (Goetz & Breneman, 1988). Recommendations for future research include investigating how school racial climate shapes student outcomes over time as well as exploring the capability for reforms in school racial climate that may produce meaningful changes in student outcomes (Wang & Degol, 2016).

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## **APPENDIX**

### **ADDITIONAL RESOURCES**

Provided below are additional resources related to assessing and improving school racial climate:

#### **Measuring School Racial Climate**

1. These student surveys can help clarify the social boundaries that exist in school.

- Early Grades:

[http://www.tolerance.org/sites/default/files/files/pdf/mixitup\\_online\\_survey\\_06.pdf](http://www.tolerance.org/sites/default/files/files/pdf/mixitup_online_survey_06.pdf)

- Middle and Upper Grades:

[http://www.tolerance.org/sites/default/files/files/pdf/mixitup\\_online\\_survey\\_05.pdf](http://www.tolerance.org/sites/default/files/files/pdf/mixitup_online_survey_05.pdf)

2. The Comprehensive School Climate Inventory (CSCI) is a nationally recognized school climate survey measuring 13 school climate dimensions. Although it does not specifically assess racial climate, the Respect for Diversity items on the Interpersonal Relationships dimension can clarify how students, faculty and staff perceive overall norms for tolerance and mutual respect for individual differences including race and culture.

## **Responding to Everyday Prejudice, Bias and Stereotypes.**

Two resources for working with students:

The following guide aims to help teachers and students in school settings handle moments of everyday bias including when and how to speak up. *How to Implement “Speak Up at School:” Respond to Everyday Prejudice, Bias, and Stereotypes*, which is a product of the Teaching Tolerance project of the Southern Poverty Law Center, Montgomery, AL 36104. A free pdf file is available online at <http://www.tolerance.org/implement-speak-up>

Help for how to develop diversity responsive schools  
The white papers cited below provide a set of school policies and practices that can maximize opportunities to learn for all students but are particularly important for enhancing the learning outcomes of students of color:

[http://www.tolerance.org/sites/default/files/general/Diversity%20Responsive%20School\\_TT%20white%20paper%20Hawley.pdf](http://www.tolerance.org/sites/default/files/general/Diversity%20Responsive%20School_TT%20white%20paper%20Hawley.pdf)

## **FIFTEEN YEARS BEYOND MY AAPP GRADUATION**

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The African American Professors Program (AAPP, now the Grace Jordan McFadden Professors Program) training that focused on the importance of developing and documenting teaching, scholarship, and service activities when building a case for tenure and promotion were always at the forefront of my mind as I began a career journey as a tenure track Assistant Professor of English in 2002. Fifteen years later, as a Full Professor of English, the AAPP training continues to influence how I manage my career in the academy as well as the mentoring I provide to others who cross my path. In this monograph chapter, I provide a narrative of pivotal moments since I graduated from the

University of South Carolina (USC) and AAPP fifteen years ago. The intent of this narrative is to demonstrate that the decisions I made during my professional journey were highly informed decisions that would not have been possible without the training and mentoring provided by AAPP.

## **JOURNEYING TOWARD COURSE DIVERSITY**

In 2002, I began working for Southern Polytechnic State University as an Assistant Professor of English. I was the only full-time tenure track African American in the Department of English; therefore, there were no other African Americans in my department to ask for advice or mentoring. For the most part, my new colleagues were welcoming. I received many tips about how to manage the teaching load, which was, at that time, four courses per semester.

My initial teaching load consisted of four composition classes for the first and second semester. I was supposed to dedicate most my energy to the first-year composition program; I quickly learned that teaching four composition



courses was a challenge. Since I was teaching full-time for the first time, I had no idea what it would be like to manage multiple sets of student papers. I realized that the way I had been trained to teach composition would not serve me well because of my four-course teaching load. During graduate-school training, I was taught to provide students with extensive editorial comments in the margins and detailed end comments for both initial and final drafts. The method of grading I had been taught worked well when teaching only one or two composition courses per semester; however, it did not work well when teaching four composition courses. There was also a bit of pressure from the composition program members to assign writing on a weekly basis and somehow to evaluate it in a timely manner; therefore, I knew I would need to determine an efficient manner of grading that was a true representation of my teaching philosophy.

After completing my first academic term, I used my summer break to begin developing teaching methods that would be more effective. I decided I to employ the Charles Cooper method of grading, which I remembered being

exposed to in Rise Axelrod and Charles Cooper's *St. Martin's Guide to Writing, Instructor's Resource Manual* (1991). This method of evaluation allows the instructor to focus on particular areas in a draft rather than the whole draft, and it offers strategies for managing the grading assessment of large sets of essays. I also began using a grading rubric to provide students with feedback. Before using the rubric, I was reading and marking students' papers by hand. For example, I would write comments in the margins and provide editing suggestions as well. Often, I would spend 20 to 45 minutes per five-page student paper. Grading was a daunting task, and I knew that I was not serving myself or my students well by providing their feedback in this manner. Once I began using a rubric and the Charles Cooper Method (1991) of grading, I started to feel good about teaching composition. In addition to using rubrics, I made time for feedback sessions with my students; those feedback sessions felt genuine, and they allowed for reciprocal learning. As I helped students shape their content and ideas in this manner, I also learned about their topics of personal interest.

The other challenge I faced during my early period of being a faculty member was feeling confident enough to ask for what I needed without raising the concerns of others who might think that I was being overly assertive in my desires as a junior faculty member. Although I had begun to use the Charles Cooper methodology for handling the student feedback and grading, the teaching schedule of only composition/writing courses made it quite difficult to focus on other faculty workload requirements, which also included service and scholarship. I knew I needed a more diverse teaching load to be able to allocate enough time in my schedule for working on writing projects to fulfill the scholarship expectations that were appropriate for my faculty position.

Eventually, I worked up enough confidence to make it known to a tenured faculty member in the composition program that I would like to have the opportunity to teach other courses for the department. I sensed that there might be some concerns about revealing such teaching desires because I was hired to teach composition. Although I enjoyed teaching composition, in addition to needing a

more diverse teaching load, I also wanted the opportunity to gain experience teaching other courses. Once I gained buy-in from the faculty members of the composition program, I aimed for a course schedule that would include teaching in the technical communication degree program and teaching some of the departmental general education literature courses. My request was entered as part of a department faculty agenda, which did not raise any concerns for me until I realized that this agenda item had thrust me into the middle of a debate that obviously had taken place many times. In the subsequent faculty meeting, the conflict was centered on which faculty actually were credentialed appropriately to teach the technical writing and literature courses.

Although the department was hiring adjuncts (i.e., contracted non-faculty instructors) to teach both technical communications and literature courses, I could see that several faculty members had expressions of concern on their faces when my departmental agenda item came up for discussion. I felt a bit nervous as I could sense that I had perhaps been nudged to put my desires out in a manner that

was confrontational and was without knowledge of a departmental backstory. Several senior faculty members made it clear there were no issues with my request; however, one of the senior professors at that time reiterated that I had been hired to teach in the composition program and then made it clear that the only courses I qualified to teach according to the Southern Association of Colleges and Schools (SACS) was perhaps African American Literature and Culture. The African American Literature and Culture course was the principal territory of both of the faculty members who were part of the committee that had hired me, and it was only being offered during spring semesters.

If I believed I could be scheduled for regular sections of African American literature each term, I would gladly have made it known that I was willing to teach only African American literature in addition to my composition courses. Since that was not the case, I carefully listened to my colleagues discuss professional development plans, which I would need to consider if I wanted to teach technical communication and literature courses. Certainly,

my agenda item had been heard and a recommendation had come forth; thus, I decided without any hesitancy to do what was necessary to attain the diverse teaching schedule that I desired.

## **INTEGRATING FEEDBACK INTO A PROFESSIONAL DEVELOPMENT PLAN**

The recommendation was to participate in a 6-day intensive seminar known as the Institute for Technical Communication organized by the Two-Year College English Association (TYCA). The seminar was recommended by several senior faculty members who already had established themselves as technical communication experts through their publications and consultations. Although I strongly believed I already was qualified to teach technical communication because of my graduate training in rhetoric and composition, I also strongly believed it was important to follow the advice and mentoring of the senior faculty in the department. A doctoral degree in English with an emphasis in rhetoric and composition was still relatively new and differed from the English degrees held by most

faculty in our department; thus, some of the senior faculty believed consulting experience with technical writing and professional relationships with technical communicators in the field was a more important qualifier than my strongly related terminal degree.

In order to become qualified to teach technical writing for the department, I enrolled in the intensive technical writing seminar facilitated by a respected organizational group known as the Society for Technical Communication (STC). The seminar location was in Conway, South Carolina, near Myrtle Beach. I was able to travel both to my home state and travel to my seminar destination; therefore, it became a win-win situation. Because the seminar was taught by a well-published and experienced technical communication professor, I came away from the seminar with a binder containing potential technical writing assignments and respected articles in the field of technical communication. Subsequently, I also attended meetings and gatherings held by the STC of Atlanta, Georgia and eventually interviewed two of the technical communication professionals to develop a better understanding of the

subject matter from a practical perspective in workplace settings. One of those professionals worked for a private non-profit organization and the other one worked for a federal government agency. Attending the technical writing seminar, participating in the technical communication society, and interviewing professional technical communicators proved an excellent and meaningful learning experience; yet, most importantly, it satisfied the requirements of the senior faculty members.

In addition to the recommended plan, I had been advised to take an additional 6 credit hours of graduate level literature courses in preparation for teaching literature in the general education area of our degree programs. Although I believed that my teaching experience at that time, in addition to my doctoral, masters and undergraduate degrees in English qualified me for teaching the lower-level general education courses in American literature, I knew I needed to respect the recommendation of the senior faculty. Fortunately, full-time faculty who teach in the University System of Georgia qualify to enroll in other state supported universities and their college courses without paying for the



course credit hours; therefore, I was able to enroll in graduate level literature courses at the University of Georgia without being required to pay tuition.

While others in my department might have viewed taking additional graduate level courses in American literature possibly as off-putting, I did not view this opportunity to learn from a pejorative view. I spent a delightful number of weeks researching American literature courses that I thought would be uncontested by my senior faculty colleagues, and I eventually settled on a course focused on multicultural American literature being offered at The University of Georgia in Athens. I enrolled in the course and eagerly read the course materials, and I enthusiastically attended the weekly seminar class in order to dialogue with my fellow classmates and the professor of the course. Because I already was credentialed with a terminal degree, I felt a bit more confident in sharing my ideas, and I was more comfortable delving into additional research beyond the assigned readings in the course. By the time I completed the course, I had established a strong rapport with the professor, and he was able to offer me an

additional independent directed reading course that would be tailored to satisfy the expectations of senior faculty in my department. Furthermore, this also satisfied my thirst for studying American literature with a multicultural focus.

In addition to satisfying criteria to teach technical communication and literature courses, I also was positioning myself to teach in the online environment. First and foremost, I had a passion to make use of technology as part of my teaching pedagogy. In fact, I knew that use of technology was a natural part of my true identity. I had been engaged with technology since a TRS-80 computer had been given to me as a gift by my paternal grandmother (i.e., Mrs. Alma Foulks, a well-known house cleaning lady of Batesburg-Leesville, SC). I later earned an undergraduate cognate in computer science, independently studied learning management systems, HTML coding, and various software applications because of my attraction to technology. More recently, when one of the senior faculty members asked me to join her in experimenting with teaching in the online environment, my decision to join her was immediate. Because I was following the path and

encouragement of a senior faculty member, I was not going to receive push-back from others in the department who at that time (i.e., 2004 - 2005) often criticized faculty who were teaching online courses. That senior faculty member and I completed training the required by the University System of Georgia to teach within their eCore program. Because I was a bit more aware of how to make use of technology, I proved to be an asset to the senior faculty member with whom I had partnered in experimenting with online teaching. As a result, we had many meaningful dialogues for two to three years. Surprisingly, those dialogues somehow helped to position me as the go-to individual in the department for using the WebCT learning management system that was being using at that time. I taught two different online courses for the University System of Georgia and established meaningful networks with other faculty who eventually would write letters of support for my initial promotion to Associate Professor in 2006.

As I entered my fourth year as an Assistant Professor who was hired to teach composition courses, I had

expanded my course development and teaching offerings to include technical communication, American literature, African American literature, a Writer's Workshop, and Ethics and Communication. I also became experienced in teaching both fully online courses and hybrid online courses. Most importantly, I had satisfied the wishes of the senior faculty member who wanted me to do additional professional development to have access to the diverse teaching schedule. I also earned the faculty's respect by meeting their expectations. I did so in a way that demonstrated my willingness to earn my access to the course-teaching schedule. Many of them worked very hard to create this course schedule at an institution that placed a higher value on degrees and courses in fields of engineering, construction, and architecture than it placed on degrees and courses in the fields of humanities and technical communication.

## **ALIGNING PROFESSIONAL DEVELOPMENT AND SCHOLARSHIP**

The journey of my scholarly activities differed from my journey toward a diverse teaching schedule, yet it

mapped onto required professional development and scholarship in a purposeful manner. In the area of scholarly activities, I had a bit more control; therefore, I chose online pedagogy as an area to carve out a niche of scholarship for myself in my department. The idea of online teaching and online pedagogy were genuine professional passions. I began networking with other faculty in my field who shared interests in my passions. Even though online teaching was relatively new, there were special interest groups within my conference organizations, and I joined them. I also began writing reviews for an online journal about those special interest group meetings. The list of scholarly activities shown in Appendix A provides examples of connections between my online teaching, professional development and scholarship.

A review of the activities listed in Appendix A reveals examples of a 13-year trajectory of scholarly activity related to technology. As early as 2004, my aim was to be involved in professional dialogue about technology. Conference presentation activities resulted in positive feedback, and publications allowed for the development of

meaningful connections with others who were impassioned also about the potential for technology to transform teaching practices. The early years of positive feedback during conference presentations provided incentives to continue engaging with technology and helping institutional colleagues (who were often my co-presenters) buy-into my endeavors into the pedagogy of online teaching. Their awareness and support of my interest in online-teaching pedagogy and related scholarly activities later were articulated clearly in annual faculty performance reviews, and subsequently these colleagues provided letters of support for each step of the tenure and promotion process.

Now as a tenured Full Professor, one still can see my trajectory of scholarship related to technology unfolding and perhaps maturing. For example, as cited in Appendix A, in the fall of 2016, I received approval for two IRB research projects focused on technology. In the spring of 2017, I published my first academic blog entry as an active leader of The Leadership and Mentoring Institute, an

organizational unit of The American Association of Blacks in Higher Education (AABHE) (Carter, 2017).

## **ENGAGING IN PURPOSEFUL AND PASSIONATE SERVICE ACTIVITIES**

My service activities differed from my journey towards a diverse teaching schedule, and it was not necessary to align those activities with my professional development and scholarship in a purposeful manner. Faculty at my institution had to showcase exemplary accomplishments in the area of teaching to achieve promotion and tenure, but they were allowed to select two additional areas for exemplary achievement. The choices included the areas of service, professional development, and scholarship. While initially I did not intend to showcase service as an exemplary or a noteworthy area for evaluation, the more than 60 items of curriculum vitae accomplishments stood out as an obvious qualifying area for promotion to Associate Professor and Full Professor. What I did do purposefully that was relative to taking on service obligations was to refuse to shy away from those activities that pushed me outside of my introverted comfort zone.

The list of service activities listed in Appendix B exemplify instances that either pushed me beyond my comfort zone to strengthen interpersonal skills, or they represent instances that aligned my professional passions with institutional requirements for service.

Among the service activities that appear in Appendix B, some represent challenges to move beyond my comfort zone in order to improve interpersonal skills. For example, I served as Faculty Moderator of the School of Arts and Sciences at Southern Polytechnic State University (SPSU; Now Kennesaw State University) because it allowed me a genuine opportunity to publicly engage with faculty members in a manner that showcased my public speaking skills, writing skills, and interpersonal skills. In 2007, I was nominated by my department chair for the position of moderator because he wanted other faculty to see my leadership skills. Although I would not have willingly sought out such a role, I knew my department chair (at that moment in time) was pushing me in the right direction for my own good as an introverted faculty member. Likewise, I was nominated to be a committee member of the National



Council of English's (NCTE) Language Policy Committee by a prominent organizational leader in 2004. I accepted the nomination and became a member because I knew I needed to work with faculty from throughout the nation to strengthen my professional confidence and interpersonal skills. My service activities with the Language Policy Committee spanned more than 10 years of active and formal membership.

Regarding service in the local community, in 2009 a coveted invitation was extended, and I agreed to serve and participate actively in Georgia's Cobb County Educational Consortium. That acceptance came with many requirements to engage in team-building leadership activities and service outreach with a diverse set of professionals in Higher Education and K-12 schools. The end result of my combined and cumulative service activities allowed me to claim a local and national record of service activity, which was a significant requirement for achieving the rank of Full Professor. Furthermore, these service activities were a significant set of experiences that honed my interpersonal skills.

There are a number of service activities listed in Appendix B that connect with my faculty passions in higher education. For instance, I desired to help develop and direct my institution's campus writing center because I could personally and professionally identify with the importance of university writing centers for students. During my graduate training, I worked with the Writing Center of the University of South Carolina as well as a similar program known as The Writing Studio. I certainly always believed them to be an important area of university service in which to make a difference in the lives of students. Likewise, I provided service as my department's online coordinator because it also represents an area of passion. Being the online coordinator is an example of where my interest in online teaching pedagogy and scholarship align with both my service and professional development interests. I enjoy helping to educate faculty about the benefits of using technology in their teaching, and I also enjoy the on-going reciprocal learning that results from professional service as online coordinator.

For me, the area of faculty service proved to be an effective way to develop my interpersonal skills and challenge myself to move beyond my natural introverted tendencies, which often can be read as a lack of departmental, institutional, or professional engagement. I strategically engaged in service activities that represented genuine alignments with my faculty passions. Overall, the area of service proved fruitful during early and mid-years in the professoriate after graduating from AAPP. In part, achieving excellence in the area of service helped me to achieve tenure and promotion, and it has continued to increase my standing as a senior professor with fifteen years of service in the University System of Georgia.

## **CONCLUDING THOUGHTS: FIFTEEN YEARS LATER**

As I stated in the opening paragraph of this monograph chapter, I aimed to show that the careful and purposeful decisions made about how to succeed as a tenure-track professor do yield success. What readers and future graduates of The Grace Jordan McFadden Professors Program should know is that many of the informed

decisions I made in order to achieve the rank of Full Professor were strategic decisions due to being exposed to numerous workshops and mentoring sessions organized by this very program. My success as a Full Professor is due to the mentoring and training that I received in preparation to be a professor. The AAPP/GJMPP experience is extremely unique. I conclude by urging current program participants not to take the program's offerings for granted if they plan to pursue careers in fields of higher education: Listen, learn, and apply.

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## APPENDICES

### **APPENDIX A: Examples of Conference Presentations, Publications and Grants**

- “Technology’s Impact on Composition Courses in the 21st Century: Student Identity, Online Course Options, Electronic Portfolios and Chats, and Writing about Literature Using Networked Chats.” Two-Year College English Association–Southeast Conference, Huntsville, Alabama, February, 26, 2004
- “Time is All We Have: Using WebCT to Generate Critical Peer Responses.” 8th Annual Student Success in First-Year Composition Conference, Georgia Southern University, Statesboro, Georgia, February, 2006
- “Balancing Act: A Rationale for Teaching Composition with Online Technology,” part of a group proposal entitled “Articulating Coherent Pedagogies: Intersections of Identity and Completeness in the Composition Classroom.” 95th National Council of Teachers of English, Annual Convention, Nashville, Tennessee, November 2006
- Writing and the iGeneration: Composition in the Computer-Mediated Classroom. Published by Fountainhead Press, Southlake, TX, 2008. (Co-edited collection representing two years of reviewing, editing,

collaborating with 23 peers in the field of English studies throughout the U.S.)

- “CCCC Committee Research into Best Practices for Online Writing Instruction (OWI).” Published Review in *Karios: A Journal of Rhetoric, Technology, and Pedagogy*, 2009
- “Understanding State-of-the-Art Online Writing Instruction: Results from the CCCC Committee’s National Survey on Best Practices” Published Review in *Karios, A Journal of Rhetoric, Technology, and Pedagogy*, 2011
- “Conceptualizing the Importance of Digital Scholarship for African American Educators” Presentation at the Association of Blacks in Higher Education Conference in Long Beach, California, April, 2016
- Institutional Review Board [IRB] Study entitled “Conceptualizing the Importance of Digital Scholarship” approved September 2016.
- Institutional Review Board [IRB] Study entitled “African American Archival Research and [Digital] Publication Project” approved November 2016.
- “Mentoring Matters,” Online Publication in Leadership and Mentoring Institute Blog, March 2017.

## **APPENDIX B: Examples of Service Activities**

- Workshop Facilitator with Language Policy Committee, “Language Diversity in the Composition Classroom Workshop,” Conference on College Composition and Communication, Chicago, March 2006
- Faculty Moderator for the University’s School of Arts and Science, Fall 200 – Spring 2008
- Edited Book Collection Manuscript Reviewer, Web 2.0 Applications for Composition Classrooms. Fountainhead Press X Series for Professional Development, October 2011
- Participant in Cobb County Educational Consortium, August 2009 – April 2011
- Writing Center, Director, Fall 2012 – Summer 2015
- Panel Speaker, “The Leadership and Mentoring Institute: Ten Years of Success—Where Do We Go From Here?” ABBHE Annual Conference, Atlanta GA, March 2, 2013
- Department Online Coordinator, Fall 2015 – Spring 2017
- Chair of Department Faculty Council, Spring 2016 – Present.



# **A CASE STUDY OF MERGERS IN HIGHER EDUCATION: SHARING PURPOSE, VISION AND IDENTITY**

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A case study is defined generally as “both a method of analysis and a specific research design” through which a “problem is examined in order to generalize the findings across populations” (University of Southern California [USC] Libraries, 2017, para. 1). Case study research is an examination of “a person, place, event, phenomenon, or other type of subject of analysis in order to extrapolate key themes and results that...provide a means for understanding an important research problem with greater clarity” (USC Libraries, 2017, para. 2). A related literature review and the research methods are discussed later in this monograph chapter, and the nature of the problem that is examined through this current case study is described below using

implementation of appropriate standard ethnographic research confidentiality practices. The names and locations of individuals and organizations have been changed to protect their identity and confidentiality throughout this report of ethnographic research, which is presented here in a case study format.

## **THE CASE STUDY**

The Center for Practical Partnerships in Education (CPPE) at the University of Wolverton was created by the General Assembly in June of 2013 and was aligned with goals set forth by the Wolverton Education Oversight Committee. The CPPE is housed in the Teachers College and co-directed by Dean Scott Phillips & Dr. Andie Smith. The goal of the CPPE is to provide educational outreach services to the public schools, families, and communities in Wolverton. The CPPE is comprised of five previously independent core partners who focus on professional development, engagement, and policy research. The five core partners are the Wolverton Improvement Council, the Writing Improvement Network, the Wolverton Educational

Policy Center, the Wolverton Geographic Alliance and the Wolverton Initiative for Middle Grades.

Three of the five partners have an independent history of service to the community as well as one previous association in common. The Wolverton Improvement Council was established over 30 years ago to provide the member training, technical assistance, statutory accountability, and other resources vital to the continued success of community-based School Improvement Councils in each of the state's K-12 public schools. The Writing Improvement Network serves as a professional development resource for Wolverton Pre K-12 teachers. The network uses expert teachers to provide training in the implementation of research-based best practices in teaching students to become better readers and writers, along with providing guidance for teachers in choosing professional development and classroom resources. The Wolverton Educational Policy Center provides information about educational policy research to all citizens, interested educators, and educational policy makers throughout the local geographic region and the nation. The faculty and

staff researches policy issues related to pre-kindergarten through high school and postsecondary education, and they disseminate that information to educational decision makers at all levels. These three entities previously were associated with the Teachers College.

The Wolverton Geographic Alliance and the Wolverton Initiative for Middle Grades also share a long history, but they previously were associated with a different college at the university. The Wolverton Geographic Alliance provides Wolverton students and educators with innovative and effective practices, materials, and research to improve geography education. The Alliance advocates for an informed, geographically-literate population that will be able to participate and compete as responsible citizens within a global economy. The Wolverton Initiative for Middle Grades works through partnerships and collaborations with educators and researchers, government agencies, non-profit organizations, and post-secondary institutions to sponsor projects that support improved academic achievement and positive youth development.

## **THE RESEARCH PROBLEM**

Before the unification, these five core partners functioned in an independent manner with separate budgets. By combining these entities under one umbrella, the state legislature hoped to reduce any redundancy, to stretch the budget dollars, and to improve the overall quality of service to the state. The goal of the CPPE is to continue their current efforts with emphasis on the three main areas of concentration, which include professional development, community engagement, and educational research and policy dissemination. What initially concerned the leadership was that the five entities seem to be failing to integrate with the CPPE's shared purpose, vision and identity. The purpose of this case study was to provide an evaluative report with recommendations on how to create and nurture a shared identity based on organizational data and formative assessments. Specifically, the study was designed to provide data on how the merger process is affecting employee productivity and satisfaction through the following research questions: (a) What are employee

perceptions of the merger process? (b) Is there a difference in the perception of employees who are leaders and non-leaders?

## **LITERATURE REVIEW AND BEST PRACTICES**

Whether in the public or private sector and without regard for an industry type, mergers occur for various reasons with different degrees of impact (both positive and negative) upon a newly merged organization. Mergers often originate from strategic failures, scarcity of resources, increased competition or economies of scale (Shein, 1985). The resulting organization is likely to have tangible and intangible benefits, both of which affect future success. Tangible benefits include financial and operational aspects, while intangible benefits include culture and identity (Rowley, 1997). Research has shown that both types of benefits are needed for a successful merger (Bouchikhi & Kimberly, 2012).

In higher education, institutions have been trending towards internal and external mergers due to decreases in federal and state funding, dwindling endowments, lower

enrollment and rising administrative costs (Cai, 2008; Rowley, 1997; Sanda & Adjei-Benin, 2011). A merger of programs and even institutions has been utilized strategically to reduce redundancy, establish synergies not previously realized, manage difficult budget times as well as to ensure self-preservation (Cai, 2008; Rowley, 1997). While merging may appeal to boards of trustees and administrators, mergers create uncertainty at lower levels of the institution if there is no transparency or a lack of effective communication (Sanda & Adjei-Benin, 2011). To ensure a successful merger, management should take precautions against infighting, gossiping and unanticipated shifts or disconnects between two or more previously distinct identities or cultures (Thompson, Strickland, & Gamble, 2005). Creating a shared vision for the newly merged organization is one of the first steps to undertake (Cartwright & Cooper, 1996). Cartwright and Cooper also espouse that without a unified purpose that is accepted by employees, divisions and units, the people involved may not visualize their place or fit well within the new organization.

Best practices suggest that a stronger focus on soft organizational behavior issues like identity rather than operational issues will lead to a successful merger (Bouchihki & Kimberly, 2012; Rowley, 1997). This is not to say that one aspect is of greater importance than the other, but rather that both foci are needed for a truly successful merger. Research has shown that culture often is mistaken for identity and vice versa (Ravasi & Schultz, 2006; Schein, 1985). In general, identity correlates with who we are, while culture speaks to the way things are done. Mergers may create fear of identity loss and raise questions about the combined identity of the new organization; thus, it is important to consider the new identity, even in the pre-merger phase (Ravasi & Schultz, 2006; Bartels, Douwes, de Jong, Pryun, 2006).

Strong and consistent communication about the new identity, direction and future can aid the new organization and its executives in removing doubt and confusion surrounding the merger process. Lack of transparency is the most commonly cited issue for employees of merging organizations (Cartwright & Cooper, 1996; Harrison,



2013). According to Harrison (2013), effective communication is one of the most important issues in studies of mergers. A lack of effective communication can lead to a loss of status and influence for those involved, which also is related to the identity threats previously mentioned (Ravasi & Schultz, 2006). According to Bouchikhi and Kimberly (2012) and other researchers, employees will have greater trust in the newly formed organization if the merger process is transparent, and their input is sought. Employee engagement is highly valued and has proven to be valuable to the foundation of new mergers (Cartwright & Cooper, 1996; Thompson, et al., 2005). Executives often have made the mistake of neglecting internal audiences, and history has provided ample examples of how this neglectful environment has failed (Bouchikhi & Kimberly, 2012; Rowley, 1997; Schein, 1985). In general, merging organizations should not overlook the importance of creating a shared identity through a shared mission, shared values, open communication and a transparent yet inclusive process.

## **RESEARCH METHODOLOGY**

To better understand the kinds of issues surrounding the CPPE merger of this case study, data from both CPPE leaders and employees were gathered using mixed methods. Since this study was an evaluation of a merged organization and not intended as true empirical research, convenience sampling was used to select the study participants. In organization studies, the use of convenience sampling for selecting participants is a better alternative than statistically-based probability sampling, since it allows for the theoretical generalization of the findings (Bryman, 1989; Calder, Phillips, & Tybout, 1981; Mohammad, Habib, & Zakaria, 2010). The selection of the participants was characterized by a sense of snowballing (Patton, 1990; Sanda, 2010) derived from the researchers' criteria that persons to be selected for the study (i.e., data sources) must be willing participants who have lived through the CPPE merger process. As a result, all 16 employees across the five entities were invited to participate as study participants.

The study began with phone interviews with the seven CPPE leaders (two administrative faculty charged with oversight of the newly merged organization and the five directors of the merging entities). Email invitations were sent for each phase of the study, seeking the CPPE's voluntary participation. All seven CPPE leaders were interviewed by phone using a uniquely designed instrument with four core open-ended questions followed by a free-form interaction period. Interview questions addressed the benefits and drawbacks of the merger, individual versus shared identity, and what is needed to make the transition more successful. Specifically, the following questions were asked:

- What are the benefits of a unified entity such as the CPPE? Are there any drawbacks?
- How is effectiveness and success measured within the program? Have any of the goals changed since the creation of the CPPE?
- Do you feel it's more important to retain former identities or create a new CPPE identity? Do you feel as if you have lost your identity?

- What could be done to make this transition more successful?

Each interview lasted about 15-20 minutes and focused on the leaders' perspective of the merger process. Interview results were transcribed and categorically coded in order to draw out common themes.

In the second phase, a 38-item self-administered questionnaire entitled "CPPE Transition Survey" was used as the survey data collection tool. The first three items in the questionnaire were demographic measures. The remaining 35 questions were adapted from Sanda and Adjei-Benin's (2011) survey instrument on employee satisfaction with the organizational change process. The questions were posed on a 1-5 Likert-type scale ranging from strongly disagree (1) to strongly agree (5) that covered the following organizational behavioral categories: Communication, engagement, identity, job satisfaction, and leadership support. As such, these 35 questions, which were associated with one another, are multiple indicators of the same construct (i.e., employees' satisfaction with their

organization's merger). This association provided the questionnaire with both construct and convergent validity. For each question in the questionnaire, response options were provided to which the respondents selected the option applicable to them. The questionnaire had a brief synopsis that explained the purpose of the study to the respondents.

The survey was administered to all sixteen CPPE employees (N=16), whose positions ranged from graduate assistant to CPPE leadership. The survey instrument was adapted from Sanda and Adjei-Benin's 2011 research study and was deemed reliable based upon previously reported Cronbach Alpha's of 0.60 to 0.84 for all five subscales. The CPPE Transition Survey was conducted electronically using JotForm, a web-based survey platform that was open to all CPPE employees for 21 days. The reliability of the questionnaire was tested by piloting it among six experts (i.e., academic faculty and staff) at the University of Wolverton. This helped check the stability, readability and consistency of the items in the questionnaire. Their feedback was used to make minor edits to the questionnaire items. The survey generated a sample size of 12 for a

response rate of 75%. Given the small population and sample sizes, there was not enough data generated to reproduce the Cronbach Alpha measure of reliability.

Research from the field of higher education states that it is better to put less focus on the nature of the changes caused by a merger and to emphasize the members' perception of the changes, as this can be the greatest source of employee stress (Arnolds, Stofile, & Lilah, 2013). This logic seems to apply to the CPPE as well. Bartels and colleagues (2006) suggest that identification of an organization is contingent on similarity between the individual and group, and the focus on change and continuity also serve as factors affecting identification. Siehl and Martin (1998) and Cai (2008) all suggest that research findings are heightened when the use of both qualitative and quantitative measures are used to study the post-merger process. Based on these literature review findings, the study was conducted using both methodologies.

## **DATA ANALYSIS AND FINDINGS**

### **Interview Data Collection**

The leaders within the CPPE participated fully in the interview process and shared valuable information for the study. As expected, there were some commonalities and differences in the responses that will provide insight to the core issues of the merger from each individual's perspective. Conceptual content analysis was used to analyze the transcripts of the five structured interviews. The interview data were analyzed using conceptual content analysis. The researcher used Carley's (1993) eight steps of conceptual content analysis. These steps included:

1. Decide the level of analysis.
2. Decide how many concepts to code for.
3. Decide whether to code for existence or frequency of a concept.
4. Decide on how you will distinguish among concepts.
5. Develop rules for coding your texts.
6. Decide what to do with "irrelevant" information.
7. Code the texts.
8. Analyze your results.

Content analysis revealed similar answers related to benefits such as collaboration, improved efficiencies, and natural synergies established in the merger (see Table 1).

One CCPE leader mentioned the benefit of natural commonality between his network and some of the others. The fit would allow for increased collaboration and efficiency. For example, in classrooms today, reading and writing curriculum often are introduced together. The creation of the CPPE allows for two organizations like the Wolverton Initiative for Middle Grades and the Writing Improvement Council to work in a collaborative, synergistic manner. Before the merger, this type of collaboration did not exist.

The interviews also revealed some drawbacks or disagreements that relate to identity threats, confusion related to the merger, and compatibility concerns (See Table 1). Multiple leaders mentioned the CPPE was a threat to their current identity. Some of the members felt some level of threat to their individual identities, which clearly seems to show that the merger has not been



**Table 1.** Content Analysis from Interviews

<b>Interview Questions</b>	<b>Issue</b>	<b>Frequency</b>
Benefits of CPPE	Funding	3
	Collaboration	4
	Efficiency	1
	Identity	2
Drawbacks of CPPE	Identity	2
	Lack of Compatibility	3
	Confusion	2
Success Measurement	Speaking with one voice	1
	Systematic Data Collection	1
	Goals Changed After Merger	4
	Goals Have Not Changed After Merger	3
Identity	Retain old identity	2
	Create new identity	4
Suggestion for Improvement	Communication / Awareness	4
	Find commonalities	1
	Workshops	1
	Hire integration professional	1

successful in fostering a shared identity thus far. Some leaders also shared confusion and a lack of understanding as to why merging into the CPPE was necessary. They also shared concerns of whether members believe they are compatible with the vision and purpose of the other entities. Of the seven leaders that are associated with the CPPE, four believed that a new CPPE identity should be established, and the rest were either opposed to a new identity or unsure.

Establishing a new identity has both positive and negative ramifications. If the CPPE were to retain their existing identities, the relationships already established throughout the state may not need changing, whereas a new identity may require time to re-brand relationships because of changes in goals and strategies. Conversely, if the CPPE does not capitalize on the natural synergies that exist, establishing an even stronger identity may prove to be difficult. Regardless of whether each leader believes in establishing a new identity, the CPPE will have a new identity that is shaped by their efforts going forward. The continuity of working together appears to be of the utmost

importance when deciding identity. Collaboration was mentioned as a perceived benefit; thus, the move towards shaping a new and stronger identity most likely is attainable.

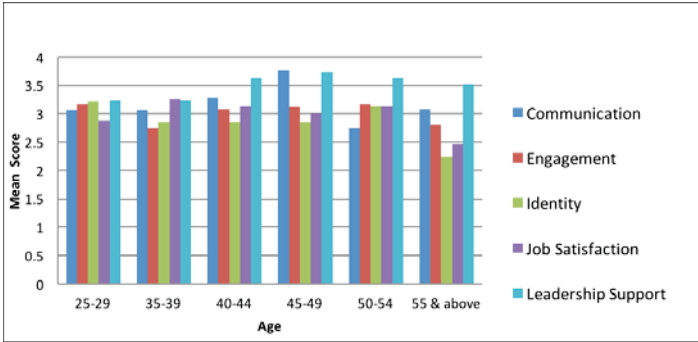
An overwhelming response from the CPPE leadership agreed that communication and awareness would have made the transition more successful. Through the interviews, the researcher learned of a planned retreat for the members of the CPPE, which is considered a good first step to addressing the lack of communication perceived by all. Additional communication of potential strengths and opportunities for improvement may have helped with the concern of threats to identity, lack of commonalities, and natural synergies, as well as to point out the benefits of the merger.

### **Survey Data Collection**

Aggregated survey results contain analyzed demographics and five categories of organizational behavior (i.e., communication, engagement, identity, job satisfaction, and leadership support). The average results,

according age demographics, are shown in Figure 1 below. In this figure, mean scores for each age grouping were originally color coded; however, in the black and gray toned figure below, the order of the content bars (reading from left to right) for each age grouping are in the order listed on the right side of the figure (i.e., communication, engagement, identity, job satisfaction, and leadership support

**Figure 1.** Mean Organizational Behavior Score by Age Group



support). These data show that CPPE’s employees believe more leadership support exists, especially among the older demographic. Table 2 displays the average scores of each category broken down by employee group: (a) Total

Average, (b) Leadership Average, and (c) Non-Leaders Average.

**Table 2.** Mean Scores of Organizational Behavior by Employee Type

Org. Behavior Employee	<i>Com- muni- cation</i>	<i>Identity</i>	<i>Engage -ment</i>	<i>Leader- ship Support</i>	<i>Job Satisfac- -tion</i>
Total	2.761	2.397	2.977	3.471	2.814
Leader	2.750	2.175	2.983	3.545	2.706
Non- Leader	2.770	2.583	2.972	3.409	2.895

Overall, identity had the lowest average score for all employee groups. Conversely, leadership support is the strongest category, which signifies a strong employee-supervisor relationship. The remaining three categories of communication, engagement and job satisfaction all

reported average mid-range responses, between 2.5 and 3 on the scale of 5.

For each category, the researcher identified what outliers existed, if any, between the leaders and non-leaders. The threshold for outliers were the top and bottom 10% of questions that have the largest gap between average leadership scores and average non-leaders' scores. This is important because it represents questions that expose potential disconnects between leadership and non-leaders. In summary, the survey reflects higher scores from the leadership than from non-leaders regarding the transparency of the merger process; thus, non-leaders do not see the process as being transparent. The results also indicate that the leaders believe that they have adequate information about the merger and that the process is clear and understandable. Not surprisingly, non-leaders' responses do not lean strongly in this direction.

Similarly, the researcher identified categories that were most agreeable among leaders and non-leaders. Commonality exists in the communication and leadership support categories. Notably, the averages for these

questions are all well above the means for the individual categories as illustrated in Figure 1 above. Of course, without the benefit of a true statistical analysis it is not possible to tell if this trend is significant. Leadership appears to believe strongly that the transition is transparent, clear and understandable, whereas the non-leaders believe otherwise. Employees strongly agree that they have not received complete information about the merger and are left confused, whereas the leadership does not agree with that statement.

### **Interpretation of Results**

The results seem to indicate a large disconnect between the leadership's vision of the merger and that of non-leaders. With employees seeming to be pointed in different directions, the CPPE may need to focus on helping all employees share in the same purpose, vision, and identity that should have been established through the merger process.

Based on the interview data and survey analysis above, two main issues surface for discussion. The first issue is

identity (i.e., whether the entities involved retain their pre-merger identity or develop a new one), and the second issue is the lack of communication and the existence of disconnects between leadership and employees. Clearly, the goal for CPPE leadership is to strengthen the already established benefits of the merger and to diminish the perceived drawbacks.

## **CONCLUSIONS AND APPLICATIONS**

Currently, the five entities that comprise the CPPE previously had their own purpose, vision, and identity, but there is evidence that they are failing to share in their new CPPE's purpose, vision, and identity. These issues have been explored through interviews, surveys, and outside research. Based upon these data, the researcher recommends a series of strategic initiatives to provide impactful results that address the CPPE's current organizational issues. As the CPPE moves forward with their integration plans, the following recommendations should be included in their post-merger process: (a) to develop and execute a communication plan, (b) to utilize an integration partner,



and (c) to implement the use of success metrics. These recommendations are based upon the research findings and supported by the literature review and best practice research (e.g., Bouchikhi & Kimberly, 2012). The organization also would benefit most likely from addressing the initial problem statement and incorporating the key findings revealed in this data analysis into their planning.

### **Improving Communication**

One of the major issues with creating a unified identity for the Center for Practical Partnerships in Education (CPPE) is the lack of communication. Effective communication starts at the top of the organization, and for the CPPE, the responsibility falls directly on the shoulders of Dean Philips. While it is true that there is a CPPE website that brings awareness to the state educational community, there is a lack of informational synapses within the internal CPPE framework. Communication is said to be the life-blood of any organization, but it apparently is especially

important for the CPPE, which is currently in a post-merger phase.

A common concern of the five CPPE executives is the lack of knowledge pertaining to what each individual group does. One way to formally enhance awareness at all levels of the new organization would be for the dean's office to publish a monthly bulletin that addresses the CPPE. The bulletin might contain current accomplishments and future activities for a particular member of the CPPE or for the CPPE as a whole. This could either be in print form to be placed in a common area to spurn conversation, or it could be given a direct, wide-distribution via email. While the general attitude of the bulletin needs to be positive and upbeat, the periodical would have higher readership and impact if it also contained pertinent extrinsic updates on pending education legislation at a national and state level. Another feature of the newsletter would be publicizing the common concerns of their customers (i.e., the Wolverton teaching and parent community). As the members of the CPPE currently operate in a segregated environment, this type of awareness presents the opportunity for regular

dialogue and for well-informed strategy development to occur.

Another method that could be used to foster internal dialogue in the CPPE would be open and public recognition of the successes of the CPPE outside of the organization, as recommended by researchers (e.g., Arnolds, et al., 2013). Again, this is an effort that should be initiated by the dean, which would be a sounding board for all of the collective achievements of the members of the CPPE. The benefits are twofold; first, it allows for the non-CPPE affiliated educational community to be aware of the mission and objectives of the CPPE. These non-CPPE educational community members might range from students and professors of the Teachers College to the Wolverson educational community or to the Wolverson State legislature. Secondly, the public recognition of the CPPE's success would act as a form of positive reinforcement. Both of these benefits would encourage a positive perception and would alleviate any fears of uncertainty regarding the purpose of the CPPE.

Finally, a more direct form of communication would be initiated to establish both formal and informal feedback loops. Some examples of the surface level or cosmetic changes associated with a merger might be a change in name, logo, or financial structure; however, a deeper-rooted transformation takes place within the leadership and governance structures of the new organization. Consistent monitoring of the changes of the organization's collective identity could be achieved by setting regularly scheduled meetings with all of the members or by encouraging individual impromptu meetings with the dean and any of the five executives. If candidness is a concern, the dean may want to consider anonymous surveys as well. As mentioned before, this would allow the dean to receive key updates on the status of the merger, but subconsciously, it also exhibits openness and willingness to engage the stakeholders in a meaningful way.

### **Integration Partners & Strategy**

As mentioned previously, the five educational outreach organizations have been brought together into one

organization for financial and governance purposes under Dean Philips as an extension of the Teachers College. While this union largely has been political and has surface level advantages, there are some deeper benefits to this merger that have yet to be realized. One of the most important benefits is the underlying purpose of work that unifies all of these organizations; of course, that work is to serve the educational communities of Wolverton by providing best practices, contextual workshops, and an outlet to voice concerns. For the past ten months, these aforementioned organizations have had little traction in understanding what synergies may be realized. It should be noted that many of the members of the CPPE hold other positions and have other obligations outside of the CPPE. As a result, organizational issues within the CPPE often can go unaddressed for large spans of time. In conjunction with the over allocation of essential resources, the lack of intentional leadership and direction provided by the dean to forge these groups together has hindered developmental progress.

A great deal of energy has been spent to sell the value of the CPPE to the external Wolverton Educational community. As that happens, the internal audiences of the CPPE members can become neglected. Employee engagement suffers if it is not intentionally addressed at the CPPE employee member level. The lack of employee engagement can suffer if it is not addressed intentionally at the CPPE member level. The lack of employee engagement was revealed as a recurring issue throughout the study. To help increase this engagement, the CPPE should employ some form of integration strategy, which could be delivered via an integration partner. After taking an assessment of the current progress and expected outcomes of the merger, the integration partner would work with the dean and the five member organizations to design a customized plan that would achieve the envisioned synergies beyond the legal and financial realm.

In order to execute an integration strategy and ensure that the reformation of the new entity's identity takes shape, it is important to understand the dynamics of forming groups or teams. When joining a new team,

members must balance and then adapt their needs for uniqueness or distinctiveness with their needs for inclusion and importance (Bartels, et al., 2006). Bartels, et al. indicate that only when the benefits beyond the surface level have been conceived properly will there be an opportunity to alter the desire for a shared identity. Furthermore, prior individual allegiances to business partners (i.e., specific school districts, educational boards, other nonprofit organizations) become less of a consideration, and the emphasis of effort then shifts to supporting the mutually conceived vision and scope of the merged organization (i.e., in this case, the CPPE) (Knippenberg, 2000). More simply put, the conception of a unified identity is the exact transition point from when a group's focus turns from "me" to "we," and the group transforms into a team. The motivation behind this change is not benevolence, but rather to gain from each other mutually (Bouchikhi & Kimberly, 2012).

Subsequent to these steps being taken, the CPPE members will then begin to invest or engage in the idea of the new CPPE. Displayed behaviors will align most

naturally with their new beliefs, and among these new behaviors would be the open exchange of best practices or self-motivated investigation of mutual opportunities for cooperation. Finally, these behaviors can be influenced by coaching and positive reinforcement, if deemed necessary. It is also worth noting that this type of metamorphosis is not an overnight process, and the pace can be encouraged by the leadership's chosen integration partner but is strongly dictated by the team (Bouchikhi & Kimberly, 2012).

### **Success Metrics**

One of the largest issues with mergers delivering their perceived benefits or synergies is the actual implementation and monitoring of developments (Arnolds, et al., 2013). As mentioned in the previous sections, an important part of change management through a disruptive process like a merger is the continuous application of setting expectations, framing the desired behavior, the observance of the actual behavior and then giving feedback to the appropriate participants. This governance structure has not



been established at the CPPE. A measurable, objective-based, success matrix is recommended to evaluate the effectiveness of each CPPE member. While the individual members each have slightly different areas of focus and size, there probably should be a mix of customized and universally applicable key performance indicators in use.

Each measurable objective would have a target level of performance and example behaviors described. This matrix would be designed to assess how each partner is contributing to the shared mission and purpose of the CPPE; thus, if a member entity is performing at or above expectations, it is operating within the desired shared identity. Such an approach will be useful in the future, especially for when additional organizations are being evaluated for inclusion into the CPPE.

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## **EUMELANIN-INSPIRED BIINDOLES VIA SOLVENT-FREE CONDITIONS**

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Nature can be a great resource for designing new materials for various applications. Creating nature-inspired compounds can translate into durable, long-lasting sustainable molecules for numerous purposes, including applications within electronic devices. Melanin is a biopolymer macromolecule found in hair, eyes, skin and feathers (Solano, 2014), and it actually acts as a natural sunscreen, providing protection from the harmful radiation of the sun (Beer & Hearing, 2007; Brenner & Hearing, 2008) by dissipating 99% of absorbed ultraviolet and infrared radiation (Brenner & Hearing, 2008). In general,

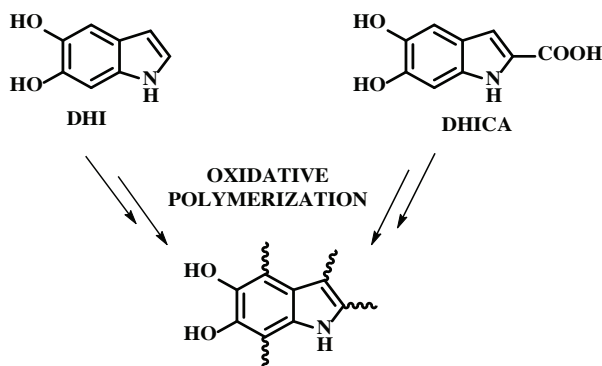
melanin may be regarded as one of the most primitive pigmentary conjugated macromolecules found in natural organisms and in living systems.

Melanin is derived from the ancient Greek word *melanos* meaning dark (Solano, 2014), and it is known to have several functions in the human body. Berzelius, a Swedish chemist first applied the term Melanin in 1840 to refer to a dark pigment extracted from eye membranes (Berzelius & Wöhler, 1840; Borovansky, 2011). Melanin is divided into three major types: Eumelanin (black-brown), Pheomelanin (brown-red) and Allomelanin, with Eumelanin being the most predominant (Solano, 2014; Simon & Peles, 2010). In the human body, melanin executes numerous specific roles including acting as a photo protectant in skin and eyes as well as being responsible for hair and eye color (Rees, 2003). It also helps with neurotransmission in the *Substantia Nigra* (i.e., Latin for black substance; Fedorow, Tribl, Halliday, Gerlach, Riederer, & Double, 2005) area of the brain. Structurally, Melanins are known generally to be a class of very diverse, complex and undefined pigments.

Over the past 60 years, several structural definitions have been proposed but most have been disproven due to their lack of demonstrating Melanin's wide variety in color, size, occurrence, and functions (d'Ischia, et al., 2013; Nicolaus, 1968; Prota, 1992a). While the exact structures are unknown, a widely accepted structural description "of all types of Melanin would be a heterogeneous polymer derived by the oxidation polymerization of phenols and their resulting quinones" (Solano, 2014, p. 2). In addition, Eumelanin is the most predominantly studied Melanin in the field of material science (Prota, 1992b; Solano, 2017)

Eumelanin can be defined as the black-brown variety of Melanin and is formed by the oxidative polymerization of the two building blocks (i.e., 5,6-dihydroxyindole [DHI] and 5,6-dihydroxyindole-2-carboxylic acid [DHICA]) shown in Figure 1. Extensive research has been done on the optical, electronic, physical, metal chelating, and structural properties of natural and synthetic Eumelanin (Corani, et al., 2014), and the structure of monomers of Eumelanin (Figure 1) is our depiction of the oxidative polymerization process of the previous research. In Francisco Solana's

**Figure 1.** Structures of two monomers that make up Eumelanin.



2014 review article, he indicates that DHI is “the most abundant” unit present in natural Eumelanin, but DHICA also is present in lesser quantities.

Ground breaking work by McGinness and Proctor on electrical switching established Eumelanin as amorphous organic semiconductors (McGinness, Corry, & Proctor, 1974); thus, Eumelanin has an excellent broad-light absorption, ranging from 200 nm to 700 nm in the electromagnetic spectrum, electrical conductivity reaching



$10^{-5} \text{ Scm}^{-1}$ , and it exhibits good charge mobility (i.e., movement of negative or positive charges) as high as  $2.1 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  (Crippa, Cristofolletti, & Romeo, 1978). As a result of these key properties, Eumelanin has been proposed for organic electronics, photovoltaics as well as for memory devices (Meredith, Tandy, & Mostert, 2013); however, these Eumelanin materials also have poor solubility and produce thin, brittle films with poor morphologies.

## **THE RESEARCH QUESTION**

The focus of our research question was whether synthetic Eumelanin biindoles could be produced by a mechanochemical process, which possibly would open doors for potential applications to bioelectronics and biocompatible implantable devices for clinical applications. In this monograph chapter, we report the solvent-free synthesis of Eumelanin-inspired biindoles as a novel approach toward the development of synthetic Eumelanins, which can have numerous potential applications in industry. The research project was designed as a

mechanochemical experiment based on previous research conducted by our team.

Mechanochemistry can be defined as achieving chemical reaction by any mechanical force (Beyer & Clausen-Schaumann, 2005). More specifically, Maria Burke (2011) states that it is “the interplay between mechanical and chemical energies” (para.1) like rubbing, grinding or milling “to bring about chemical reactions” (para. 1) some of which were being used in prehistoric times. Burke credits Laszlo Takacs, University of Maryland Associate Professor of Physics and Director of the UMBC NanoImaging Facility, as advocating a more modern definition that is less straightforward. He is quoted as saying that “it is best to define mechanochemistry broadly, as the branch of chemistry dealing with the chemical effects of mechanical action...especially in inorganic and metallurgical systems, [and] the most important direct effect is ‘activation,’ meaning the creation of fresh surfaces...” (Burke, Modern Terminology, para. 1). Some current research focuses “on investigating mechanochemistry to design polymers with improved properties”

(Burke, 2011, Future Directions, para. 1). As noted by Do and Tomislav (2017), “green chemistry” is a major underpinning of the rediscovery of mechanochemistry, which is a means of enabling chemical transformations to be achieved in solvent-free environments. This chemistry can eliminate the need for solvation of starting materials in the process of creating synthetic eumelanin; therefore, conventional stirring and heating would not be necessary (Beyer & Clausen-Schaumann, 2005). Our grinding and sonication methods are described in the methods section that follows.

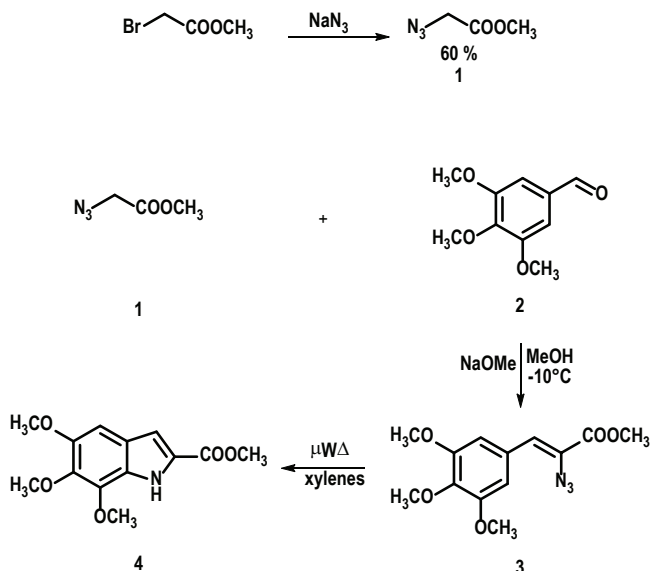
## **METHODS**

The synthesis of synthetic Eumelanin begins with obtaining an Eumelanin-inspired starting material. The grinding and sonication methods described here increase the probability for molecules to collide, activating the chemical reaction (Jordens, Appermont, Gielen, Van Gerven, & Braeken, 2016). To assure adequate safety procedures, protective equipment was worn at all times

during the experiments in which our personnel worked; thus, researchers wore lab coats, goggles, and lab gloves.

To obtain an Eumelanin-inspired starting material, the Hemetsberger indole synthesis was used according to the reported research by Wang (2009) and is shown in the top row of Figure 2. In our experiments, commercially available methyl bromoacetate (5 g, 26 mmol), which is shown at the top left of the figure, was reacted with sodium azide (2 g, 32 mmol) in methanol (40 mL) to produce methyl 2-azidoacetate (shown at the top right as compound **#1**; 3.67 g, 60 % yield). In the middle row of Figure 2, the previous prepared methyl 2-azidoacetate, shown as **#1** (5 g, 41 mmol), was reacted with the commercially available compound, 3,4,5-trimethoxy-benzaldehyde, shown as **#2** (2 g, 10 mmol) under basic conditions (NaOMe, 2 g, 41 mmol) in 20 mL of methanol. That reaction produced the azidoacrylate product, which is shown as **#3** (3 g, 37 % yield). This resulting azidoacrylate, which is **#3** (50 mg, 1.7 mmol), was cyclized using microwave heating (expressed as  $\mu\text{W}\Delta$  in the third row of Figure 2) in xylenes (5 mL) to

**Figure 2.** Synthesis of Eumelanin-inspired starting material.



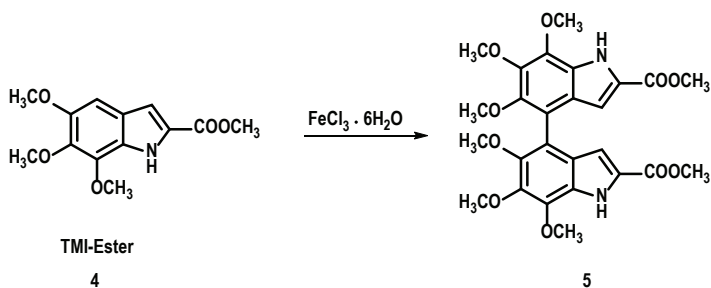
produce the Eumelanin-inspired starting material, methyl 5,6,7-trimethoxy-1*H*-indole-2-carboxylate (20 mg, 54% yield) that is shown as #4 in Figure 2 (bottom left). From this point forward, the Eumelanin-inspired starting material, methyl 5,6,7-trimethoxy-1*H*-indole-2-carboxylate will be expressed as TMI-ester.

Even though common synthesis of Eumelanin generally involves the chemical or enzymatic synthesis of these molecules or dopamine in solvent, here we present a “green” solvent-free method for synthesis of Eumelanin-inspired biindoles by ultrasonic waves (Figure 3). It also was an automated mechanochemical process in which physical grinding was not needed, and the frequency of collisions was controlled. In such mechanochemical processes, friction and heat activate the chemical reaction (Jordens, et al., 2016), but the experiment begins with creating starting materials.

In this study, the starting materials, which are TMI-ester (25 mg) and iron (III) chloride hexahydrate (50 mg) were weighed onto weigh paper on an analytical balance. Two solvent-free methods were used (i.e., a grinding method and an ultrasonication method): First, in the grinding method, TMI-ester and chloride were transferred into a mortar. Using a mortar and pestle, the TMI-ester and iron (III) chloride were ground together for four minutes, and a small sample was removed at one-minute intervals to test for reaction completion. Second, in the Ultrasonication

method (See Figure 3), TMI-ester and iron chloride were transferred into a sonication-safe vial. The vial was clamped, placed in a sonication bath, and ultrasonicated for

**Figure 3.** Synthesis of Eumelanin-inspired Dimers by Ultrasonication



an hour. Subsequently, a qualitative analysis procedure (i.e., Thin Layer Chromatography) was used to examine the results of the synthesis of Eumelanin process.

Thin Layer Chromatography (TLC) is a method for qualitative analysis of substances based on the separation of mixtures (Mohammad & Tiwari, 1990), which was introduced in the 1950s utilizing a TLC plate (Liquid Chromatography, Gas Chromatography [LCGC] Editors, 2011). The analysis reveals the presence or absence of a substance and its purity.

All types of TLC have both a stationary and a mobile phase, and quite often UV light is used (Clark, 2007). In implementation, this technique uses a stationary phase composed of a silica, or alumina on plastic, aluminum or glass backing that is called a TLC plate (Wonderhowto, 2008). This process is described by the LCGC editors (2011) as possibly being automated, but it is typically a manual technique. A drop of the compound mixture can be dropped onto the TLC plate at the starting point. This method is known as “spotting,” and the resulting drops are the “spots.” Typically, the TLC plate is transferred in a TLC chamber filled with a small amount of solvent or solvent mixture, which is referred to as the mobile phase (Mohammad & Tiwari, 1990). In this process the mobile phase wicks up the TLC and interacts with a spotted compound(s), which is an action that also is known as “developing.” How well the compound mixture interacts in the mobile and stationary phases determines how far the compound mixture travels up the TLC plate. When the mobile phase has wicked up the TLC plate for 10 cm (i.e., a finish line), the TLC plate is removed and spots are



examined. The TLC plate is considered “developed” at this point.

The mechanochemistry process in our research can be described as a redox reaction. Redox reactions are defined as “reactions in which one species is reduced and another is oxidized” (Washington University, 2005, Redox reactions, para. 1). Of course, both species must change (i.e., one atom gains an electron and the other loses an electron). In our experiments, the iron (III) chloride serves as an oxidant and converts to iron (II) chloride after one electron transfer (Sarhan & Bolm, 2009). The TMI-ester is reduced in the reduction-oxidation (redox) reaction, producing a “radical cation” on the TMI-ester. The radical cation reacts with other TMI ester radicals to form the biindole compound. During this redox reaction, the yellowish orange mixture turns a dark brown-black color. Conversely, for the grinding method, the solid reaction mixture became a sticky black paste over time because the mixture was hygroscopic (i.e., absorbs moisture from the air). As a result, the reaction would not proceed to completion, and some starting materials remained. Because the

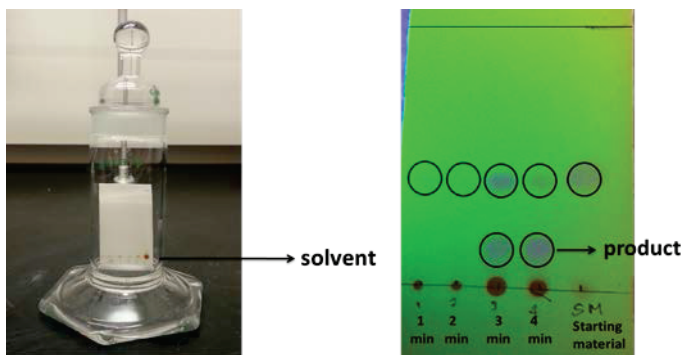
ultrasonication method was a sealed reaction; however, it was not open to the air. It also was an automated process; therefore, no physical grinding was needed, and the frequency of collisions was controlled by altering the sonication speed. In addition, the typical frequency was above 20 kHz in our study.

## RESULTS

The successful creation of the Eumelanin-inspired biindoles was accomplished only by use of ultrasonic waves, and the completion was verified at the conclusion of the chemical reaction analysis. To analyze exactly when the chemical reaction was complete, a small sample from the reaction mixture was transferred into another test tube. Ethyl acetate was used to dissolve the reaction mixture. The dissolved reaction mixture then was transferred to a silica TLC plate, using a capillary tube where a drop of the dissolved reaction was transferred onto a TLC plate as a small dot (Figure 4). The TLC plate was developed using 9:1 hexanes: ethyl acetate mixture as mobile phase in a glass TLC chamber. To monitor the reaction completion,

we spotted the starting materials versus the reaction mixture. The TLC plate was viewed in nature-white light and under Ultraviolet (UV) light (Shown in Figure 4, right). The TLC plate displayed the reaction as complete at 4 minutes of grinding or ultrasonication. Very little starting material remained at 4 minutes, according to the TLC plate of the ultrasonication process. It is noteworthy that a new spot (i.e., our biindole product) appears as a lower

**Figure 4.** Thin layer chromatography for the monitoring of reaction completion



blue spot (labeled “product” at the right). Subsequently, the desired product also has been characterized further by

structural determination methods like nuclear magnetic resonance (NMR) and mass spectroscopy.

## **DISCUSSION AND CONCLUSION**

It is clear that our research concluded with the development of a solvent-free method for the synthesis of Eumelanin-inspired biindoles. Furthermore, our procedures have demonstrated that a “green” solvent-free method such as ultrasonication can be used for synthesis of Eumelanin-inspired molecules while reducing solvent waste. These resulting Eumelanin-inspired biindoles have extensive characterization and numerous potential applications.

This approach creates a new pathway to the green syntheses of well-defined Eumelanin molecules, which opens the door for potential applications to bioelectronics (e.g., Wunsche, Cicoira, Graeff, & Santato, 2013) and biocompatible implantable devices for clinical applications. Specifically, this approach is compatible with the need for safe, clean and efficient transformations for the pharmaceutical and chemical industries (Do & Tomislav,

2017), which could open doors for more research on various applications. Do and Tomislav view the future outlook for mechanochemistry research as needing to move from the “qualitative, often only intuitive interpretations of mechanochemical reactivity,” into developing “a quantitative mechanistically well-understood area of chemistry” (p. 16), which will require development of new “instrumentation, precise theoretical models, and extensive systematic studies” as well as furthering the understanding of how all these new experimental techniques “can be used to control reactivity” (p. 16). Since such research developments are likely to stimulate and accelerate growth within the field of mechanochemistry (Do & Tomislav, 2017), our research will continue in synchrony with this growing area of advancement in chemistry research.

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# **EVALUATING THE ACCURACY OF COGNITIVE ABILITY ESTIMATES**

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High school educational testing programs provide test takers with scores weeks, sometimes, months after the test administration. With an increase in computer-based testing, some test developers want to reduce the amount of time in which a test taker can receive a score. One way to be able to offer test takers a faster delivery of scores is to offer computer-based tests that can be scored within minutes after the exam is taken. “In theory, computer scoring can be faster, reduce costs, increase accuracy and eliminate concerns about rater consistency and fatigue” (Anglin, Anglin, Schumann, & Kaliski, 2008, p. 57). Test takers often are interested in receiving scores faster to help with future decision making and planning. Other stakeholders want to receive scores quickly because the results may be

one of many sources the decision maker will use to determine college admissions, subsequent course taking, and even teacher incentives. Given the high stakes of the decisions associated with many high school examinations, an accurate and precise interpretation of scores is encouraged (American Educational Research Association [AERA], American Psychological Association [APA], and National Council on Measurement in Education [NCME], 2014).

Stakeholders who use these scores want to be sure the scores from different administrations may be interpreted in the same way. For example, if one test taker takes a college admission exam in May and another test taker takes a different form of the college admission exam in October, the stakeholder interpreting the scores from these exams would like to be able to compare the scores from the two test takers with strong confidence that the scores have the same meaning (AERA, APA, NCME, 2014).

Given the importance of the decisions that are made for high school students using cognitive ability measures or national standardized tests, the processes of development,

scoring and reporting of scores for the tests typically are implemented through a collaboration of several teams designated to ensure the integrity of the measure (AERA, APA, & NCME, 2014). The phases of developing high school tests for a national or a state administration include the design of the test, writing of the test questions, evaluating the test questions for bias towards special protected populations, pre-testing of the test questions, development of multiple test forms, administering the test, scoring the test questions, and reporting the results to various stakeholders (AERA, APA, & NCME, 2014).

Psychometricians are members of the test development team who are responsible for ensuring the accuracy and precision of the scores test takers receive across multiple administrations for the same exam. There are several methods psychometricians can use to ensure accuracy and precision of test scores. Given the need to make immediate scoring decisions for computer-based tests, psychometricians will rely less on the common practice of analyzing the data in several different ways and choosing the most appropriate result after the test administration. The

demand for computer-based testing, encourages psychometricians to analyze all of the possible ways in which test takers of varying levels of cognitive ability may respond to test questions and choose the best scoring method prior to test administration. Making such decisions before the test administration allows for development of a scoring algorithm that can be executed on demand when a test taker completes the computer-based test.

Developing a scoring algorithm for a high stakes computer-based tests is a complex procedure, which provides psychometricians with incentives to examine thoroughly various decision points that may impact the accuracy and precision of the estimation, commonly referred to as the root mean squared error and the variance of an estimate, respectively (Walther & Moore, 2005). Some of the scoring decisions that may impact an ability estimate include the number of items on the test, the quality of the test items, the choice of the probability model, the choice of the estimation method, and the choice of prior distributions for the estimation method (Kolen & Tong, 2010; Magis, Béland, & Raïche, 2011).

The purpose of this study is to compare and contrast the bias, accuracy, and precision of three of the most commonly used methods for determining a test score or estimating the examinee's cognitive ability level when the number of items on the test varies. In particular, this investigation examines the bias, accuracy, and precision of the ability estimates achieved when using a three-parameter logistic statistical model (3PLM) from item response theory with maximum likelihood estimation (MLE), expected a posteriori estimation (EAP), and maximum a posteriori estimation (MAP) methods for tests with lengths of 5, 10, 15, and 20 items.

## **ITEM RESPONSE THEORY**

Item response theory is comprised of several statistical models that allow the user to calculate the probability that a test taker with a specific ability level will correctly respond to a question (Yen & Fitzpatrick, 2006). The choice of a statistical model is based on the type of question and the number of points that are possible to earn for a correct answer. Most cognitive ability tests have multiple-choice

questions, which allow the test taker to earn one point per question. Some cognitive tests contain questions that require the test taker to provide a short answer or write an essay. A test taker may earn more than one point on the short answer and essay questions, which often are scored using a rubric to describe the meaning of each possible score point.

The focus of this study is the 3PLM, a statistical probability model that is most appropriate for test questions that are dichotomously scored (i.e., test questions that have two possible score options, correct or incorrect; Yen & Fitzpatrick, 2006). The 3PLM in item response theory allows for the psychometrician to estimate the probability that a test taker with a certain ability level will respond correctly to an item based on the relationship between the following characteristics of the test questions: (a) the difficulty of the test questions, (b) the extent to which the test items discriminate between test takers or differentiate the ability levels of the test takers, and (c) the extent to which the test takers guessed when responding to the test



questions as well as the cognitive ability of a test taker on the measured content. The formula for the 3PLM is:

$$P_j(\theta) = c_j + (1 - c_j) \frac{\exp(1.702 * a_j(\theta - b_j))}{1 + \exp(1.702 * a_j(\theta - b_j))}$$

where  $a_j, b_j, c_j$  are the discrimination, difficulty, and guessing parameters for item  $j$ , respectively.

## ESTIMATION METHODS

When using an estimate (i.e., a measure that contains a true value and some error) to support high-stakes decisions, psychometricians often use more than one method. There are two major families of point estimation methods, which are known as non-Bayesian and Bayesian. The methods within each family have some advantages and disadvantages that a psychometrician must consider when selecting an estimation method for a testing program. The MLE method, a non-Bayesian method, uses an algorithm to find the maximum ability level where a test taker has the possibility of achieving the same set of scored responses (i.e., correct and incorrect answers), given the value of each

of the item parameters and the model (Yen & Fitzpatrick, 2006). The MLE can be found using the following equation:  $L(\theta) = \prod_{j=1}^J P_j(\theta)^{Y_j} Q_j(\theta)^{1-Y_j}$ , where  $P_j(\theta)$  is the probability of a correct response to item  $j$  and  $Q_j(\theta) = 1 - P_j(\theta)$  is the probability of an incorrect response to an item  $j$  (Magis, Béland, & Raïche, 2011).

Some psychometricians choose the MLE method because the estimates are consistent, have minimum standard errors, and will remain unbiased as the number of items increase (Magis, Béland, & Raïche, 2011; Yen & Fitzpatrick, 2006). In addition, the estimates from the MLE method do not depend on the variety of ability levels of the test takers (Kolen & Tong, 2010). There are two major drawbacks of the MLE method: (a) the method is unable to produce an ability estimate for test takers who correctly respond or incorrectly respond to all questions on the test, and (b) the method may identify more than one local maxima for the three-parameter logistic model (Magis & Raïche, 2010).

One primary advantage of the EAP and MAP is the ability to produce an estimate for test takers who correctly respond or incorrectly respond to all questions on the test (Yen & Fitzpatrick, 2006). EAP and MAP methods are similar to the MLE, but the algorithm allows the user to provide additional information (also known as a prior distribution) about the performance of similar test takers on a related test or from a previous test administration, or from a prior distribution. The algorithm for the EAP and MAP is the prior distribution,  $f(\theta)$  that maximizes the posterior distribution,  $g(\theta)$  that is equivalent to  $f(\theta) * L(\theta)$  (Magis, Béland, & Raïche, 2011). The EAP method uses the mean of the score distribution from similar examinees to inform the estimates, and the MAP uses the mode of the score distribution from similar test takers to inform the estimates (Yen & Fitzpatrick, 2006). For testing programs with a long history of administrations, psychometricians are able to calculate accurately the mean and mode of prior distributions; as such, the standard errors for the EAP and MAP methods are smaller than those of the MLE (Yen & Fitzpatrick, 2006).

# BIAS, PRECISION AND ACCURACY OF ESTIMATES

Bias, precision, and accuracy are core concepts of estimation theory that help the user to interpret the quality of estimation. Walther & Moore (2005) describe bias, precision, and accuracy in the following ways: (a) bias is the distance between a population measure and the true value of the measure; (b) precision is the variance of an estimate; and (c) accuracy is the combination of bias and precision or the distance between the estimated value and the true value. Highly desirable estimates are those that are unbiased, contain minimal variance and are quite close to the true value.

An unbiased estimate is one in which the value of the overestimates is equal to the value of the underestimates; thus, the bias is equal to zero (Walther & Moore, 2005). Psychometricians often examine the bias, precision (standard error or variance) and the accuracy (root mean squared error or mean squared error) at individual score points across the ability distribution by using the following formulas:

$$\text{Bias}(\hat{\theta}) = \sum_{r=1}^N (\hat{\theta}_r - \theta) \quad ,$$

$$SE(\hat{\theta}) = \sqrt{\frac{1}{N} \sum_{r=1}^N \left( \hat{\theta}_r - \frac{\sum_{t=1}^N \theta_t}{N} \right)^2} \quad \text{and}$$

$$RMSE(\hat{\theta}) = \sqrt{\frac{1}{N} \sum_{r=1}^N (\hat{\theta}_r - \theta)^2}, \text{ where } \theta \text{ is the true ability,}$$

$\hat{\theta}_r$  is the estimated ability for the  $r$ th replication, and  $N$  is the number of replications. In the Kolen & Tong (2010, p.10) study of proficiency estimates, they suggest the following relationship is expected to hold when the

distribution of  $\theta$  is well specified:  $var(\hat{\theta}_{MLE}) \geq var(\hat{\theta}_{EAP})$ ; where  $var(\hat{\theta}_{MLE})$  is the variance of the ability estimate based on the MLE method and  $var(\hat{\theta}_{EAP})$  is based on the EAP method.

## **SIMULATION STUDY DESIGN**

To examine the bias, precision, and accuracy of ability estimates across various estimation methods, data were simulated for test lengths of 5, 10, 15, and 20 items. The discrimination, difficulty, and guessing parameters for the 3PLM were randomly chosen from the following normal distributions:

$$a \sim N(1, 0.2^2), b \sim N(0.1), \text{ and } c \sim N(0.2, 0.01^2).$$

The true ability estimates were selected from the following values of -4, -3, -2, -1, 0, 1, 2, 3, and 4. Using the random item parameters and the selected ability estimates as input for the 3PLM, item responses for 1,000 test takers were generated at each of the nine ability levels. This process was then replicated 1,000 times for each test length. The randomly generated item response files were used as input for calibration and scoring in FlexMIRT software (Cai, 2017). The results from FlexMIRT provided item parameter estimates and ability estimates for each of the test takers.

## **RESULTS**

### **Descriptive Statistics**

Table 1 presents the descriptive statistics for ability, discrimination, difficulty, and guessing parameters for the true or baseline condition and the average over all of the 1,000 replications for each test length. The average over all replications was quite close to the baseline measures. This indicates that the data were well simulated.

**Table 1.** Simulation study descriptive statistics

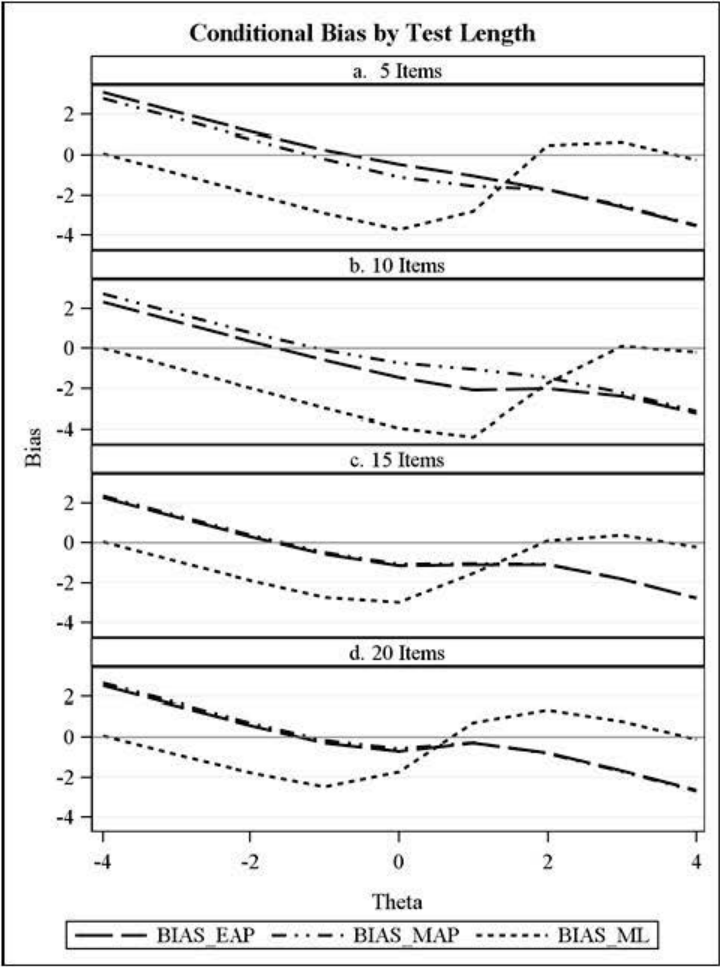
	True Statistics		Estimates	
	Mean	SD	Mean	SD
Ability( $\theta$ )	0.00	2.58	0.00	1.00
Discrimination ( $a$ )	1.00	0.40	0.98	0.39
Difficulty ( $b$ )	0.00	1.00	0.00	1.00
Guessing ( $c$ )	0.20	0.10	0.20	0.11

### Conditional Indices

Figures 1–3 show the bias, standard error and root mean squared error conditioned on ability distribution for all test lengths (5, 10, 15, and 20) and estimation methods (EAP, MAP and ML). The bias in Figure 1 is performing as expected with some of the values overestimating and some of the values underestimating. As the test length increases the bias estimation for the ML method actually improves.

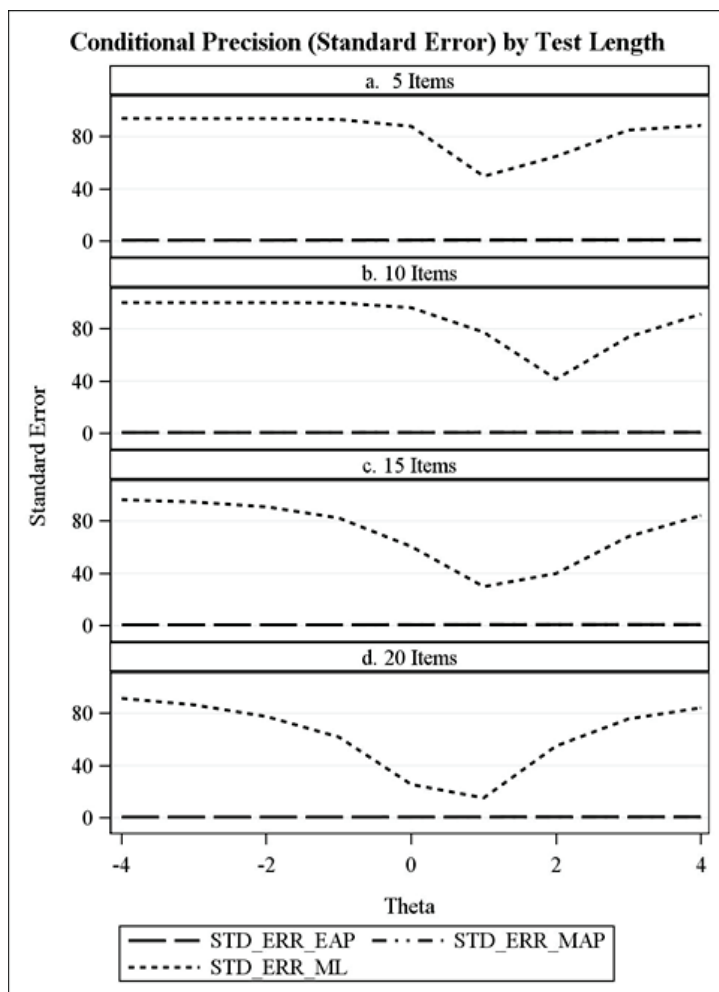
The standard error in Figure 2 is showing the largest amount of variability for the ML method for all test lengths. As the test length increases, there is a decrease in

**Figure 1.** Conditional Bias by Test Length



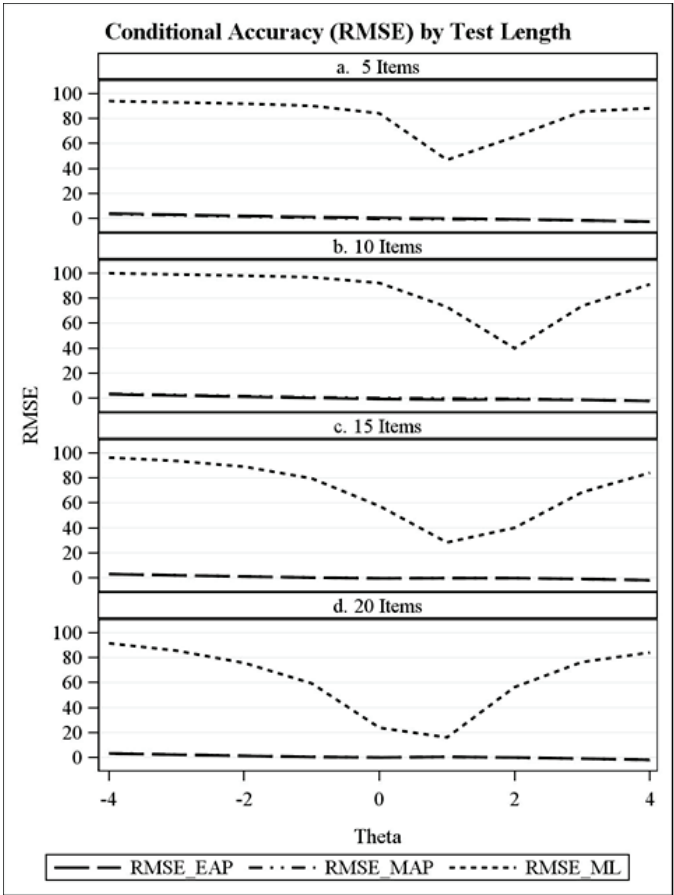


**Figure 2.** Conditional Standard Error by Test Length



standard error and root mean squared error for the ML method between theta values of 0 and 2 (See Figure 3).

**Figure 3.** Conditional Root Mean Square Error by Test Length



## **DISCUSSION AND CONCLUSION**

Given the variety of cognitive tests that are used in high schools, understanding and maximizing the accuracy of a measure of cognitive ability is paramount when the result may be one of several information sources for making high stakes decisions for these high school students. In addition to understanding and maximizing the accuracy of the measure, psychometricians also want to reduce the timeline for delivery of test scores. A reduction in the timeline for delivery of scores may be possible with the development of scoring algorithms implemented by the computer. The future integrity of computer based testing depends on psychometricians finding and correctly implementing the most accurate estimation method that works best for the design of the testing program.

This study explored the impact of test length (i.e., 5, 10, 15, and 20 items) on three ability estimation methods (EAP, MAP, and ML). The methods were evaluated by examination of the bias, accuracy and precision of the ability estimates. Item responses were simulated for the four test lengths using the 3PLM and normally distributed

item parameters for 1,000 test takers at each true ability level. True ability levels were integers that ranged from -4 to 4. The process was replicated 1,000 times for each test length. The ML method seemed to have the most variability. With the addition of the MAP method, the results in this study seem to support the findings of Kolen & Tong (2010), which suggest the Bayesian methods (EAP and MAP) have smaller error variance than ML estimators  $var(\hat{\theta}_{ML}) \geq var(\hat{\theta}_{MAP}) \geq var(\hat{\theta}_{EAP})$ . The reduction in the variance may be due to the quality of the prior distributions in Bayesian estimation (Kolen & Tong, 2010; Lord, 1986).

The exploration of test length in this study supports other findings, which suggest the accuracy and precision improve as the test length increases (Tong & Kolen, 2010). Because this study did not employ all of the available estimation methods, future studies probably should examine additional estimation methods such as weighted likelihood, bootstrap and jackknife methods, which were found to be less biased than the ML method. In particular, the weighted likelihood method had a smaller bias and was

computationally efficient (Warm, 1989). The bootstrap and jackknife methods were computationally intensive but were also less biased than the ML method (Wang, Jiao, & Xiang, 2013). Additional studies also should evaluate other test characteristics that may have a statistical impact on the bias, accuracy, and precision of an ability estimate. Some of these methods may include but are not limited to the types of items (e.g., short answer, essay). The combination of findings from this study and future studies can help psychometricians in delivering accurate scores within a timely manner.

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## **\*AUTHOR NOTES**

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2. The views and opinions expressed in this article are those of the author and do not necessarily reflect the official policy or position of The College Board.



# **NEONATAL KERATINOCYTE SPHEROIDS ARE ENRICHED FOR EPIDERMAL STEM-LIKE CELLS**

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The “Human papillomavirus (HPV) infection is the most common viral infection of the reproductive tract” (World Health Organization [WHO], 2016, Overview, para. 1) and is the cause of many squamous cell carcinomas, carcinomas, principally cancer of the cervix (WHO, International Agency for Research on Cancer [IARC]

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Working Group (2012, p. 255, 1.1.1). “All papilloma-viruses belong to the *Papillomaviridae* family, which includes 16 different genera” (WHO, IARC Working Group, 2012, p. 255, 1.1.1) “and can be further subdivided into high- and low-risk types depending upon their degree of association with human malignancy” (p. 255, 1.1.4). Cervical cancer remains the leading type of cancer in women and is the second leading cause of death in women worldwide (Clifford, Smith, Plummer, Muñoz, & Franceschi, 2003; WHO, Women’s Health, 2013). Infection by high-risk HPV types is relatively common, and they are a causative factor in invasive cervical cancer (Gravitt, 2011); however, cervical cancer is comparatively rare. In large part, the rarity of cervical cancer is attributed to the 90% clearance of all HPV infections within two years of onset (Burd, 2003; Hariri, Dunne, Saraiya, Unger, & Markowitz, 2014). Even though only about 10% of women infected by oncogenic HPV will develop persistent infections, only a fraction of these infections actually will progress into cancer. Although HPV persistence is a

primary cancer risk factor, Cervical cancer risk also involves cell-specific factors.

HPV infection typically occurs in the stratified epidermis, which is a stratified multilayer tissue maintained by constant renewal of suprabasal keratinocytes (Gravitt, 2011). “During the process of epidermal renewal, regeneration is governed by a putative population of epidermal stem cells (EpSCs) that are found in the basal layer, which is the regenerative compartment of the skin interfollicular epidermis (IFE) (Blanpain & Fuchs, 2006)” (Woappi, 2016, p. 4). In this basal layer, EpSCs stain positive for basal cytokeratins, are small in size, and they express high levels of integrin alpha 6 (ITG $\alpha$ 6), which are factors that mediate their attachment to the skin’s basement membrane (Barrandon & Green, 1987; Coulombe, Kopan, & Fuchs, 1989; Jones & Watt, 1993; Tani, Morris, & Kaur, 2000).

In the IFE renewal process, basal EpSCs are primed for activation apparently by environmental cues, and they divide to generate rapidly proliferating transit-amplifying cells (TAC). “The proliferating TAC subsequently leave

the basal surface and initiate a stepwise squamous differentiation program, which replenishes lost suprabasal layers (Blanpain & Fuchs, 2006)” (Woappi, 2016, p. 4). Blanpain and Fuchs indicate that activation of basal EpSCs is characterized by the co-expression of basal cytokeratin 14 and the proliferative transcription factor P63; however, the upward movement of EpSCs to suprabasal layers is associated with a loss of the basal cytokeratins as well as a gradual decrease of P63 protein levels. During the suprabasal differentiation commitment process, epidermal keratinocytes increase in size and amplify cellular epidermal growth factor (EGF) by signaling through the induction of the EGF receptor (EGFR) (Miettinen, et al., 1995; Nanney, Magid, Stoscheck, & King, 1984; Takeuchi, et al., 2001). In the later stages of differentiation, the keratinocytes completely lose P63 expression, and they also begin to express high levels of several other genes involved in the formation of the cornified envelope (Hsu, Li, & Fuchs, 2014; Pellegrini, et al., 2001).

In the conclusions of recent studies, epidermal renewal typically has been attributed to a discrete population of

basal stem cells (Clayton, Doupé, Klein, Winton, Simons, & Jones, 2007; Doupé, et al., 2012; Redvers, Li, & Kaur, 2006), but whether the IFE is being replenished hierarchically by a single cell population that can switch between quiescence (i.e., reduced proliferative activity) and activation or whether IFE regeneration is being carried out stochastically by several groups of committed progenitors (CP) is still being debated by researchers (De Rosa & De Luca, 2012; Hsu, et al., 2014; Sada, et al., 2016).

In normal epidermal tissue, the stepwise regeneration of the IFE is regulated tightly by cell attachment to the basement membrane; therefore, cell-surface detachment of normal human keratinocytes (NHKc) results in terminal differentiation, which is followed quickly by suspension-induced cell death (i.e., anoikis) (Green, 1977; Wakita & Takigawa, 1999). “This phenotypic response also is accompanied by marked induction of EGFR expression (Wakita & Takigawa, 1999). Studies by our group and others have demonstrated dramatic increases in EGFR drive keratinocyte proliferation but are poorly tolerated by

NHKc” (Woappi, 2016, p. 5) (e.g., Akerman, et al., 2001; Bheda, Creek, & Pirisi, 2008; Takeuchi, et al., 2001). Lower expression of cell surface EGFR by keratinocytes was found to be associated with spheroid formation, quiescence, and also has been attributed to epidermal stem/progenitor-like cells (Fortunel, 2003; La Fleur, Johansson, & Roberg, 2012; Sun, Goderie, & Temple, 2005; Vollmers, Wallace, Fullard, Höher, Alexander, & Reichelt, 2012). Other researchers conducting keratinocyte stem cell (KSC) studies employed EGFR cell surface levels and the cells’ spheroid-forming abilities as an assessment of keratinocyte stemness (Le Roy, et al., 2010; Shamir & Ewald, 2014; Vollmers, et al., 2012; Yu, et al., 2006). Even though recent reports indicate that spheroid culture can improve the potency of skin cells (e.g., Borena, et al., 2014; Higgins, Richardson, Ferdinando, Westgate, & Jahoda, 2010; Kang, Kwack, Kim, M., Kim, J., & Sung, 2012; Vollmers, et al., 2012), a thorough investigation of the regenerative effects of spheroid culture on (NHKc) has not been conducted.

## THE RESEARCH PROBLEM

In preliminary research, a 3-D spheroid culture model system was employed to investigate the spheroid forming ability of 59 different NHKc strains that were isolated from individual neonatal foreskin explants. Only 40% of all NHKc strains were found to be capable of aggregating into multicellular spheroids, and 60% of strains failed to form viable spheroids when cultured in suspension. In addition, spheroid-derived (SD) NHKc were observed displaying considerable cellular heterogeneity and expressed a marked induction of stem cell reprogramming factors within a 24-hour suspension culture. “When transposed into 2-D monolayer culture, we found that SD-NHKc readily restored keratinocyte colonies that stained positive both for nuclear P63, basal cytokeratin 14, and were enriched for a basal stem/progenitor subpopulation of ITGα6<sup>high</sup>/EGFR<sup>low</sup> cells. Furthermore, adherent spheroids expressed a transcriptome signature corresponding to ectoderm commitment and epidermis reconstitution” (Woappi, 2016, p. 6). NHKc spheroid cultivation was shown to be an efficient method of enriching epidermal skin

stem/progenitor-like cells from neonatal skin; therefore, this method provides a powerful new tool for the investigation of epidermal basal stem-cell activation and HPV-driven neoplasia in culture.

“In addition to HPV persistence, host-cell specific factors also contribute to cervical cancer risk” (Woappi, 2016, p. 1). Based on the current review of related literature, the target cell of HPV infection is still unknown; however, it is clear that virus particles must gain access to the epidermal basal layer of skin tissue to initiate an infection. The entry into this layer of cells results in division of basal cells that constitute the proliferative compartment of the epidermis. Longworth and Laimins (2004) believed most cervical cancers arise within the cervical transformation zone where tissue stem cells are presumed to reside; therefore, the main target cells for HPV infection are speculated to be keratinocyte stem cells (Stanley, 2006). “In recent years, it has been demonstrated that keratinocyte stem cells have sphere formation ability and express putative epidermal stem cell markers such as low levels of EGF receptor (EGFR) and elevated levels of



adhesion protein Integrin alpha 6 (INT $\alpha$ 6), which is expressed primarily on the basal membrane of the epidermis” (Woappi, 2016, pp. 1-2), which Yoon, Kim, Park, and Cheong (2001) also identify as the main receptor of cell entry for HPV16. Furthermore, keratinocytes with high levels of EGFR or low levels of integrin alpha 6 (INT $\alpha$ 6), in contrast, were found to acquire a more differentiated phenotype, yielding a poorer growth potential in culture (LeRoy et al., 2010; La Fleur, et al., 2012).

In more recent studies, some researchers purport that “the expression of HPV oncogenes in keratinocyte stem cells in vivo promotes abnormal mobilization of these cells and contributes to cancer initiation” (Woappi, 2016, p. 2). Furthermore, Michael, Lambert, and Strati (2013) also speculate that such epidermal stem cells most likely are providing a reservoir of latently infected cells that are quite likely to persist for a long time. The subsequent question that arises is focused on the investigation of “the relationship between the presence of basal stem cells in an epithelium and the susceptibility of that epithelium to HPV-mediated transformation” (Woappi, 2016, p. 2).

Consequently, this research project was designed to examine the relationship between the stem cell properties of normal human epidermal skin cells in culture as well as the susceptibility of these cells to transformation by human papillomavirus type 16 (i.e., “the etiological agent of approximately 5% of all known human cancers”; Woappi, 2016, p. 2). It was hypothesized that the stem cell properties of cervical epithelium or genital skin cells could influence their susceptibility to transformation by the DNA of the HPV virus.

The investigation of this hypothesis involved an innovative use of 3-D suspension culture and fluorescence activated cell sorting (FACS), utilizing successfully purified epidermal stem/progenitor cells from neonatal foreskin tissue. After conducting extensive immortalization experiments using these enriched populations, these cell populations were found to be more susceptible to transformation by oncogenic HPV16 DNA than the mass-cultured cells that were collected from the same individual donors. The findings were expected to provide experimental evidence that basal stem cell-like keratinocytes can

be transformed preferentially by HPV16 DNA. The methods and results that follow are drawn from the doctoral dissertation of the first author of this monograph chapter (i.e., Woappi, 2016) under the supervision of the second author.

## **MATERIALS AND METHODS**

### **Cell Culture**

To produce the cell culture, NHKc were isolated from neonatal foreskin as previously described (Akerman, et al., 2001) and were cultured in keratinocyte serum-free medium (KSFM) supplemented with 20 ng/mL EGF, 10 ng/mL FGF, 0.4% bovine serum albumin (BSA) and 4µg/mL insulin to promote progenitor cell activation. This medium will be referred to as KSFM-stem cell medium (KSFM-scm).

### **Spheroid Formation and Subcultivation**

Cultured NHKc ( $2 \times 10^4$  cells) were seeded into a 96-well round-bottom plate coated with a polymerized mixture of agarose (1.3%) and KSFM-scm. The spheroids were maintained for at least 24 hours in suspension prior to

harvest, and multicellular suspension spheroids then were plated into a 2-D monolayer culture and allowed to proliferate. These cells will be referred to as SD-NHKc. Proliferating SD-NHKc were detached by using the enzyme trypsin, tested again for spheroid formation. Subsequently, newly formed spheroids were plated again and transposed into 2-D monolayer culture to produce secondary SD-NHKc cultures. Cell size and morphology were determined using Lumenera Infinity 1 software (Lumenera Corporation, Ottawa, ON).

### **Clonal Growth Assay**

Cells were plated at low density (10,000-20,000 cells/dish) into duplicate 100-mm dishes and fed KSFM-scm every 4 days until ~25% confluence; subsequently, they were fed every 2 days. Cultures were serially passaged (1:100) in 100-mm dishes until cell proliferative capacity was exhausted. Cell numbers and viability were determined using Countess Automated Cell Counter (Invitrogen; Carlsbad, CA). Cumulative population doublings (PD) were calculated according to the formula:  $PD = (\log N / N_0)$

$/ \log_2$ , where  $N$  represents the total cell number obtained at each passage, and  $N_0$  represents the number of cells plated at the beginning of the experiment (procedures based on Ma, et al., 2015). Duplicate dishes were stained with 10% Giemsa stain to assess colony size and morphology.

### **Colony-forming Efficiency Assay**

Spheroid non-forming NHKc (NF-NHKc) and spheroid forming NHKc (SF-NHKc) cultures were plated at 10,000 cells/dish in duplicate wells of a six-well plate and fed once with 8 mL of medium and then incubated for 10-20 days. Cells were fixed with methanol and then stained with 10% Giemsa. Colony forming efficiencies (C.F.E) were calculated as colony number per dish divided by the original number of cells seeded. For 2D-attached spheroid culture, C.F.E was determined by obtaining the ratio of colonies generated from each seeded spheroid to the original number of cells contained within the spheroid.

## **Real Time PCR**

Given that RNA is the genetic product that precedes protein production in a cell, we isolated total RNA from cells using the All Prep DNA/RNA Mini Kit (Qiagen, Germantown, MD), according to the manufacturer's protocol. Reverse transcription was carried out with 1 µg of total RNA, using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Real-time PCR was performed, using iQ SYBR Green Supermix (Bio-Rad) and following the manufacturer's instructions. Amplicon products were validated by agarose gel electrophoresis (2% v/v). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. All samples were assayed in triplicate.

## **Immunohistochemistry**

Cells were grown on coverslips coated with polylysine until 75% confluent. The cells were then washed twice in ice-cold phosphate buffered saline (PBS), fixed with 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.5% Triton in 1%

glycine, and then blocked using 0.5% BSA and 5% goat serum for 30 minutes at room temperature to ensure that BSA peptides would bind to cellular epitopes. Samples were next incubated with antibodies against P63 (Thermo Scientific, 1:200 dilution) and cytokeratin 14 (Santa Cruz Biotechnology, 1:200 dilution) in blocking solution overnight at 4°C. Samples were then washed three times with PBS containing Tween 20 (PBST) followed by incubation with FITC-and Alexa 568-conjugated secondary antibodies (at 1:1000 dilution, Invitrogen, Carlsbad, CA). Nuclei were stained with 1:5000 dilution of 4', 6-diamidino-2-phenylindole (DAPI; Invitrogen) before cells were mounted. Cells then were observed using a Nikon Eclipse E600 microscope (Melville, NY) and a Zeiss confocal laser-scanning microscope (Munich, Germany; Dublin, CA USA).

### **In Vitro Lineage Tracing Assay**

The 2-D monolayer cultures were transfected with the pMSCV-IRES-EGFP plasmid vector, carrying the enhanced green fluorescent protein (eGFP) gene. Cells

were trypsinized and seeded ( $2 \times 10^4$  cells) as 3-D spheroid suspension cultures. After 24 hours, eGFP-expressing spheroids were plated into monolayer 2-D cell culture. Cells were tracked with a Zeiss Axionvert 135 fluorescence microscope (Dublin, CA) using Axiovision Rel. 4.5 software (available at <https://www.zeiss.com/microscopy/us/products/microscope-software/axiovision.html>). The number of eGFP-expressing cells then was determined by quantifying a calibrated pixel-by-pixel ratio between the green fluorescent image channel and the phase contrast image.

### **Fluorescent Activated Cell Sorting Analysis**

Cells  $2-4 \times 10^6$  cells/ml were stained with FITC-conjugated anti-integrin $\alpha 6$  (Abcam, Cambridge, MA) and PE-conjugated anti-EGFR (BD Pharmingen, San Jose, CA). Flow cytometry analysis was performed using a BD FACS Aria II flow cytometer (BD Biosciences, San Jose, CA).



## **Microarray Processing and Analysis**

Total RNA was isolated from mass cultures of an NHKc strain and respective spheroid rings, in triplicates, using the Qiagen RNeasy Plus Micro Kit (Qiagen, Germantown, MD), according to the manufacturer's protocol. RNA quality was assessed using an Agilent 2100 Bioanalyzer. Microarray experiments were performed using the Affymetrix platform (Santa Clara, CA). Arrays were scanned using a GeneChip Scanner 3000 7G system and a computer workstation equipped with the GeneChip Command Console 4.0 software (Affymetrix, Santa Clara, CA). Following completion of array scans, probe cell intensity (CEL) files were imported into Expression Console Software (Affymetrix) and were processed at the gene-level using Affymetrix's HuGene-2\_0-st library file and the Robust Multichip Analysis (RMA) algorithm to generate CHP files. After confirming data quality within the Expression Console Software, CHP files containing log<sub>2</sub> expression signals for each probe then were imported into the Transcriptome Analysis Console Software version 3.0.0.466 (Affymetrix) to analyze cell

type-specific transcriptional responses, using one-way between-subject analysis of variance.

### **Statistical Analysis**

Data were expressed as the mean  $\pm$  standard deviation (SD). Differences between mean values were analyzed using Student's *t*-test. Probabilities of  $P < 0.05$ ,  $P < 0.01$  or  $P < 0.001$  were considered statistically significant and indicated in the figures by \*, \*\* or \*\*\* respectively.

### **RESULTS**

Spheroid-derived keratinocytes contain an increased number of EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> expressing cells. Given that SD-NHKc intensely expressed basal epidermal markers, it was suspected that their prolonged growth potential was primarily sustained by a distinct fraction of keratinocyte stem cells (KSC). To explore this possibility, we employed FACS to purify populations of cells expressing high or low levels of EGFR and the basal epidermal stem cell marker integrin alpha 6 (ITGα6). Clearly, about 20% of the cells in SD-NHKc cultures were found to have low levels of EGFR and high levels of Integrin α6 (EGFR<sup>lo</sup>/ITGα6<sup>hi</sup>). In

contrast, EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells made up only 3% of the cells derived from mass cultured cell populations.

To determine the growth potential of the other cell populations represented in these cultures, we cultivated the EGFR<sup>lo</sup>/ITGα6<sup>hi</sup>, EGFR<sup>hi</sup>/ITGα6<sup>hi</sup>, and EGFR<sup>hi</sup>/ITGα6<sup>lo</sup> fractions from SF-NHKc mass cultures, and carried them on as monolayers. The results were that EGFR<sup>hi</sup>/ITGα6<sup>lo</sup> cells accumulated the fewest number of PD in culture (~3) and generated dividing colonies for only ~18 days in culture, while EGFR<sup>hi</sup>/ITGα6<sup>hi</sup> cells accumulated a total of 11.3 PD in culture and continued to generate dividing colonies for ~30 days. Even though EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells were slow-cycling during the first 15 days in culture, they later proliferated at a rate exceeding that of the other isolated cell fractions. Furthermore, EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells displayed the greatest growth potential of all FACS-isolated cells, and these cells generated dividing colonies for over 50 days in culture. Moreover, EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells appeared small in size and produced large holoclonal colonies (Barrandon & Green, 1987) with numerous cells

that co-expressed nuclear P63 and cytokeratin 14 (Figure 1A-C4).

**Figure 1.** FACS-sorted NHKc are enriched for a basal subpopulation of P63-expressing cells.

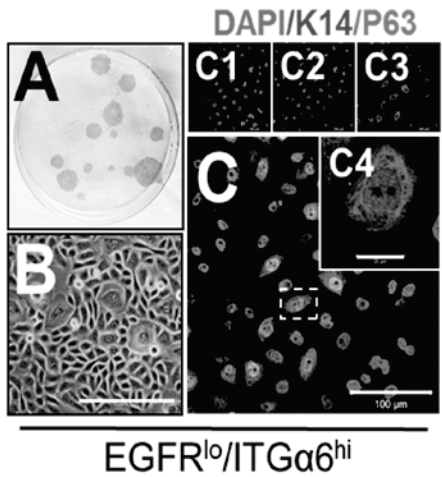


Figure 1 includes: (A) FACS sorted EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells were plated into a 6-well plate at a density of 10,000 cells/well and cultured until approximately 80% confluent. Colony density was assessed by Giemsa staining. (B) Phase contrast image of proliferating EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cell progenies in adherent culture. (C) EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cell progenies immunostained with antibodies against pan-tumor

protein 63 targeting all TP63 isoforms (P63) and basal cytokeratin 14 (K14). Scale bar=100  $\mu$ M.=

Conversely, progenies from EGFR<sup>hi</sup>/ITG $\alpha$ 6<sup>lo</sup> populations produced large elongated cells that gave rise to abortive colonies and expressed low to undetectable levels of P63 and cytokeratin 14 (Figure 2D-G). To validate the

**Figure 2.** Spheroid non-forming NHK c are enriched for a basal subpopulation of P63-negative cells.

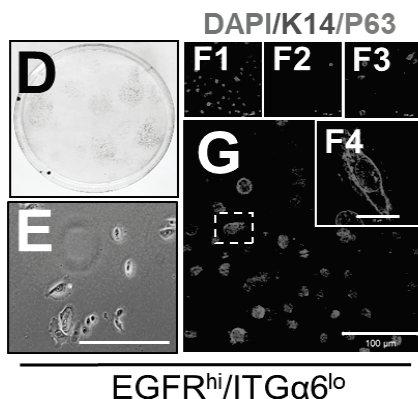


Figure 2 includes: (D) EGFR<sup>hi</sup>/ITG $\alpha$ 6<sup>lo</sup> colonies stained with Giemsa. (F1) Cells stained with the nuclear stain DAPI and for (F2) nuclear P63 and (F3) cytoplasmic keratin 14. (F4) 40 x magnification image of a single EGFR<sup>lo</sup>/ITG $\alpha$ 6<sup>hi</sup> cell progeny immunostained for nuclear

P63 (red) and cytoplas-mic K14 (green). Scale bar=20  $\mu$ M. = (G) EGFR<sup>hi</sup>/ITG $\alpha$ 6<sup>lo</sup> cells immunostained with antibodies against pan-tumor protein 63 targeting all TP63 isoforms (P63, red), and basal cytokeratin 14 (K14, green). Scale bar=100  $\mu$ M.=

expression of basal cell markers detected by immuno- staining, we performed real time RT-PCR analysis of pan- P63 and K14 cell fractions in both EGFR<sup>lo</sup>/ITG $\alpha$ 6<sup>hi</sup> and EGFR<sup>hi</sup>/ITG $\alpha$ 6<sup>lo</sup>. The PCR results revealed a 3.4-fold upregulation of mRNA encoding P63 and a 1.7-fold upregulation of mRNA encoding K14 in EGFR<sup>lo</sup>/ITG $\alpha$ 6<sup>hi</sup> progenies compared to the unsorted cell fraction. Conversely, EGFR<sup>hi</sup>/ITG $\alpha$ 6<sup>lo</sup> populations expressed undetectable levels of P63 and K14 mRNA (Figure 3H).

**Figure 3H.** FACS-sorted NHKc express increased P63 mRNA levels.

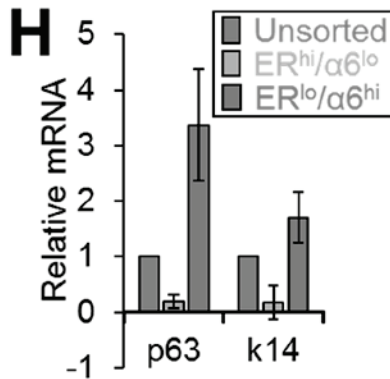


Figure 3H shows the expression of mRNAs encoding TP63 (all isoforms) and cytokeratin 14 in EGFR<sup>hi</sup>/ITGα6<sup>lo</sup> and EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells relative to unsorted population as determined by RT-PCR. Data were normalized to GAPDH expression and reported as mean +/- standard deviation.

In order to examine the spheroid-forming ability of different FACS-purified populations isolated from SD-NHKc, we seeded  $2 \times 10^4$  cells from each sorted population on 3-D soft agar cushions and found that EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells retained spheroid-forming ability (Figure 4I), while EGFR<sup>hi</sup>/ITGα6<sup>lo</sup> cells failed to form spheroids in suspension culture (Figure 4J). Furthermore, when investigating the spheroid-forming abilities of EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells purified from NF-NHKc strains, we found that they were better able to self-aggregate into multicellular spheroids as compared to the corresponding unsorted cell fractions (Figures 5 and 6).

**Figure 4.** FACS-sorted NHKc retain spheroid-forming abilities.

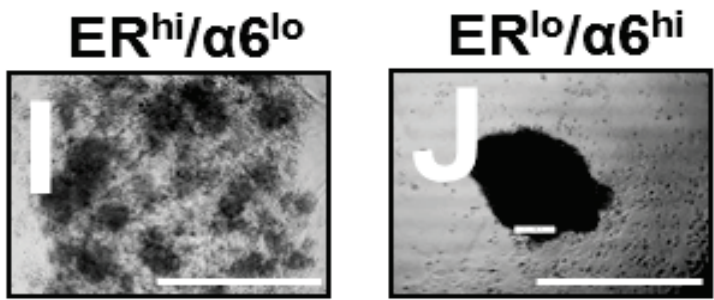


Figure 4 includes: (I) Phase contrast images of suspension cultures from both  $EGFR^{hi}/ITG\alpha6^{lo}$  and (J)  $EGFR^{lo}/ITG\alpha6^{hi}$  cells isolated from a SF-NHKc strain. Scale bar=100  $\mu$ M. =

**Figure 5.** Non-forming NHKc lack  $EGFR^{lo}/ITG\alpha6^{hi}$  cells.

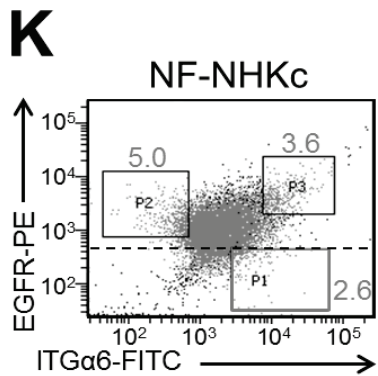




Figure 5K includes: (K) Representative flow cytometry data illustrating the gating of EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells in a NF-NHK strain, (P) Quantification of sorted cell fractions between SF-NHKc and NF-NHKc strains.

**Figure 6.** EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells differ between spheroid and non-spheroid forming cells.

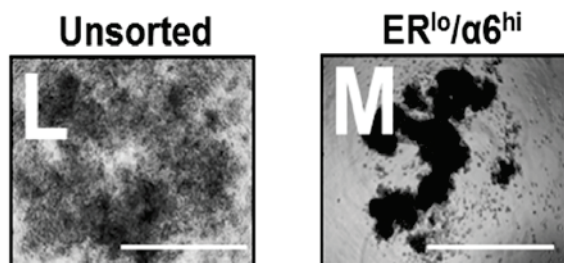


Figure 6 includes: (L) Phase contrast images of suspension cultures from unsorted populations and (M) EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells isolated from a corresponding SF-NHKc strain. Scale bar=100 μM.=

Finally, Figure 7 shows the results of quantifying the baseline proportions of EGFR<sup>lo</sup>/ITGα6<sup>hi</sup>, EGFR<sup>hi</sup>/ITGα6<sup>hi</sup> and EGFR<sup>hi</sup>/ITGα6<sup>lo</sup> cells between the SF-NHK and NF-NHKc strains; however, no significant differences between

**Figure 7.** Quantification of sorted cell fractions between SF-NHKc and NF-NHKc strains.

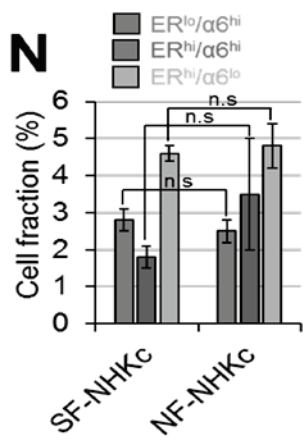


Figure 7N shows representation of FACS-sorted cell fractions from spheroid-forming and spheroid non-forming strains.

the two groups were found. Taken together, these data suggest that the presence of basal stem-like cells is a major contributing factor to spheroid-forming ability in neonatal keratinocyte cultures.

## DISCUSSION

The results of this study demonstrate that certain strains of neonatal human keratinocyte cultures, which exhibit many functional properties of EpSCs, are resistant intrinsically to suspension-induced cell death (anoikis) when cultivated as 3-D spheroid suspensions. “What makes newborn skin specimens unique for epidermis stem cell studies is their inherently greater reserves of EpSCs and the virtual absence of genomic insults that frequently accumulate in adult tissue (Gago, et al., 2009; Pirisi, Creek, Doniger, & DiPaolo, 1988; Van Der Schueren, Cassiman, & Van Den Berghe, 1980). These attributes allow for more precise investigations of factors driving epidermal cell activation in normal human skin tissue. In this study, we found that certain NHKc strains consistently formed spheroids in suspension, while others were consistently incapable of aggregating into spheroids” (Woappi, 2016, pp. 38-39).

Even though we did not find major differences in colony forming efficiencies between the freshly-isolated primary cultures from spheroid forming (SF) and spheroid

non-forming (NF) NHKc strains, it was clear from our observations that the spheroid-forming ability generally was greater in primary cultures that expressed less cell surface EGFR. Clearly, our most recent observations provide additional evidence to strengthen substantially the earlier reports by our research group that describe inter-individual variabilities in epidermal NHKc EGFR levels (Akerman, et al., 2001). These results also corroborate a number of other researchers' findings, describing EGFR<sup>low</sup> expressing NHKc as spheroid-forming basal KSC (Fortunel, 2003; Le Roy, et al., 2010).

As reported in this study, when re-plated in monolayer 2-D culture, surface-adherent spheroids were observed generating small-sized cells that traveled to produce holoclonal colonies, these colonies then fused to form epidermal sheets that stained intensely for both the proliferative marker P63 and the basal marker K14, which are indicative of TAC cells that are primed for epidermal regeneration (Fitsialos, et al., 2007; Patel, Wilson, Harding, Finlay, & Bowden, 2006).

Further probing of SD-NHKc with a plasmid transiently expressing eGFP, colony reconstitution patterns of SD<sup>GFP</sup> cells were monitored, and we found that a single SD progeny could form a fully dividing K14<sup>+</sup> colony. It seems quite likely that when spheroids attach onto a plain 2-D polystyrene cell culture dish, it is interpreted as a “loss of local confluence” (Guo & Jahoda, 2009; Roshan, Murai, Fowler, Simmons, Nikolaidou-Neokosmidou, & Jones, 2016) by keratino-cytes, which results in the triggering of colony initiation and epidermal restoration. “When conducting RT-PCR analysis on spheroids in suspension, we detected reduced mRNA levels of keratinocyte proliferation markers KI-67 and  $\Delta$ NP63, but these levels were markedly increased in SD monolayer cultures. Ingenuity Pathway Analysis (IPA) distinguished the presence of several skin cell proliferation processes activated during 2-D spheroid culture” (Woappi, 2016, pp. 39-40). The most recent data are supportive of the earlier research reported by Guo and Jahoda (2009), which demonstrates that attachment of keratinocytes onto a plain

cell-culture surface was stimulating both skin progenitor cell activation and wound-repair.

It was expected that secondary cultures from SD-NHKc would express high cell surface levels of EGFR, but in this study SD progenies were found to maintain reduced cell-surface EGFR levels and were found to be enriched significantly for a slow-cycling EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cell subpopulation. In addition, no significant differences were found in the relative proportions of EGFR<sup>lo</sup>/ITGα6<sup>hi</sup> cells between primary mass cultures from SF-NHKc and those of NF-NHKc. This finding indicates that an elevated number of basal keratinocytes is not the sole contributor to the spheroid-forming ability in primary NHKc cultures. “Incidentally, we found that spheroid suspensions expressed both basal and suprabasal markers, yet concomitantly preserved elevated expressions of mRNAs encoding pluripotent reprogramming factors Nanog, Oct-4, Sox2, and Klf4. Gene Ontology analysis revealed ectodermal commitment as a pathway significantly activated in surface-attached spheroids, indicative of the presence of a primitive cell population extant within

neonatal spheroid cultures” (Woappi, 2016, p. 40). Future research to define the commitment lineage of all cell populations within NHKc spheroids would be challenging experimentally, since the broad array of gene signatures that they would express reflects a mosaic of differentiation commitments (Mo, et al., 2013; Rybak, He, Kapoor, Cutz, & Tang, 2011).

The results of this study are pointing to two distinct cell lineages within multicellular NHKc spheroids (i.e., first, a population of basal stem/progenitor-like cells, maintaining high levels of K14 and producing holoclonal colonies in adherent culture and, second, a post-mitotic involucrin-expressing cell population that not only makes up the bulk of spheroid cultures but also eventually forms a cornified-like spheroid ring of terminally differentiated cells). “It is possible that uncommitted basal stem/progenitor populations present within primary NHKc cultures are signaled to remain in, or revert to, a less differentiated state during suspension culture, while CP are precipitated into terminal differentiation. A precise ratio between EpSCs, early TAC, and CP, therefore, may be

critical in calibrating spheroids' regenerative potency and differentiation programs in culture" (Woappi, 2016, p. 41).

To investigate the molecular factors that are underpinning these behaviors as well as to characterize further the differentiation fates undertaken by each of these cell populations would require additional studies. Our data serve to increase the evidence that progenitor cells can switch between quiescence and activation depending on microenvironmental signals and tissue regenerative needs (Doupé, et al., 2012; Horsley, Aliprantis, Polak, Glimcher, & Fuchs, 2008; Roshan, et al., 2016; Tumbur, et al., 2004). In addition, our data corroborate reports that 3-D cultivation of mammalian cells also acts as a reprogramming tool, which is capable of reverting differentiated cells to a more primitive stem state (Borena, et al., 2014; Liu, et al., 2009; Rybak, et al., 2011; Shamir & Ewald, 2014).

Regarding the hypothesis that stem cell properties of the cervical epithelium or the genital skin could influence susceptibility to transformation by virus DNA, it seems clear that a precise ratio between EpSCs, early TAC, and



CP is critical in calibrating spheroids' regenerative potency and differentiation programs in culture. Since this project was designed to explore the relationship between the stem cell properties of normal human epidermal skin cells in culture and these cells' susceptibility to transformation by human papillomavirus type 16, our results "provide experimental evidence that HPV16 DNA may preferentially transform basal stem cell-like keratinocytes" (Woappi, 2016, p. 3). Our data along with future studies "could help identify among the many people who present with persistent HVP infections, the relatively few that are truly at risk for developing cancer" (p.3).

There are many implications that emanate from our data and observations. First, insights gained from observations of the complex behaviors exhibited by primary NHKc during suspension culture include: (a) elements that influence basal cell homeostasis and (b) the broad array of cell plasticity stimulated during keratinocyte spheroid culture. Even though most EpSC investigations have been conducted in mice with a single genetic background, we believe that human newborn skin studies are a better

representation of the genetic diversity found across human specimens. “Such distinctions may have a more significant impact on mammalian epidermal studies than previously appreciated. For instance, the stark differences seen in spheroid-forming abilities between NHKc strains isolated from different individuals could be evidence of intrinsic, potentially congenital, traits influencing person-to-person propensities for basal stem/progenitor cell activation” (Woappi, 2016, p. 42). Of course, additional studies will be required to develop a better understanding of the genetic and signaling networks that regulate these behaviors and the ways they might influence pathologies that are driven by aberrant basal stem/progenitor cell activation.

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